OSSIFICATION OF THE POSTERIOR LONGITUDINAL LIGAMENT AND AGE-RELATED MACULAR DEGENERATION: A NEW AVENUE OF FORENSIC IDENTIFICATION

by

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A dissertation submitted to the faculty of
The University of Utah
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Department of Anthropology

The University of Utah

August 2013

The University of Utah Graduate School

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ABSTRACT

Anthropologists provide a valuable service to the medico-legal community. They are tasked with creating a biological profile and helping with the ultimate identification of unknown remains. This can often be a difficult task when the anthropologists require something to which they can compare the biological profile and create a small group of potential matches. This endeavor can be greatly enhanced by access to medical patient databases and identification techniques which are able to utilize specific disease databases in conjunction with the biological profile.

The research presented here explores the genetic connection between the eye disease Age-Related Macular Degeneration (AMD), and the bone disease Ossification of the Posterior Longitudinal Ligament (OPLL) as a new avenue of identification. The research conducted looked at the specific genetic marker that connects the two diseases, the interaction of this genetic risk factor with other risk genes in AMD, and the prevalence and presentation of OPLL in non-Asian populations.

The results of the project provided a great deal of insight into a possible therapeutic target for AMD and suggests that the previously reported statistics on OPLL both in, and out, of Asia are grossly underestimated, thus the disease should be reexamined in all populations throughout the world. In addition, the results and parameters of this research advocate future research into an identification method based on the genetic connection between these two diseases.

For my parents
Who gave me the ideals, encouragement, and support to accomplish any goal in life -
Even if it was playing with bones!

and

For Kar
Who always expected the best and whose enthusiasm made this research possible,
You are missed

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LIST OF ABBREVIATIONS AND TERMS

ABI SNPBrowser	Applied Biosystems SNP searching software
AIS	Adolescent idiopathic scoliosis
AMD	Age-related macular degeneration
AREDS	Age-Related Eye Disease Study
ARMS2	Age-related maculopathy susceptibility 2
BMPs	Bone morphogenetic protein family of genes
BMP4	Bone morphogenetic protein 4
C-spine	Cervical spine
C (#)	A specific vertebra in the cervical spine, numbered top-down
CDH13	Cadherin 13, H-cadherin
CFH	Compliment factor H
CLR	Conditional logistic regression
CNV	Choroidial neovascular form of AMD, or “wet” AMD
COL6A1	Collagen, type VI, alpha 1
COL11A2	Collagen, type XI, alpha 2
CT	Computed tomography
DISH	Diffuse idiopathic skeletal hyperostosis
GEE	Generalized estimating equations
GSSibs	Giuliana Silvestri Sibling Cohort

Haploview	Broad Institute software for LD and haplotype block analysis
HFE	Human hemochromatosis
HLA	Human leukocyte antigen complex (a histocompatibility complex)
HTRA1	HTRA serine peptidase 1
IGF1	Insulin-like growth factor 1
LD	Linkage disequilibrium
ME	Medical examiner
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
NCBI	National Center for Biotechnology Information: a genetic information repository and collection of medical publications
NESC	New England Sibling Cohort
NMDA	N-methyl-D-aspartic acid
NPP1	Necrosis-inducing Phytophthora protein 1
OEV	OPLL in evolution
OLF	Ossification of the ligamentum flavum
OPLL	Ossification of the posterior longitudinal ligament
OPN	Osteopontin
Pack-years	The number of packs/day times the number of years one smoked
PHI	Personal health information
PLL	Posterior longitudinal ligament
PRG1	p53-responsive gene 1
PTHR1	Parathyroid hormone 1 receptor
ROBO1	Roundabout, axon guidance receptor, homolog 1

RORA	RAR-related orphan receptor alpha
RPE	Retinal pigment epithelium
SNP	Single-nucleotide polymorphism
TGF-β	Transforming growth factor, beta superfamily of genes
TGF-β3	Transforming growth factor, beta 3
TNF	Tumor necrosis factor

ACKNOWLEDGEMENTS

This project would not have been possible without the assistance of a number of individuals. I wish to thank my dissertation committee for their support and interest in this research, especially the late Dr. Karen Ramey Burns, who was intrigued by my ideas but was never able to see the results. I would very much like to thank Dr. Dennis O'Rourke for stepping in as my committee chair and helping me refocus in a difficult time, and Dr. Michael Finnegan for bringing invaluable expertise to the committee when we lost Kar.

I would also like to thank Dr. Margaret DeAngelis for her support of the project and use of her lab facilities and patient populations. It was an eye opening experience in genetics. I would not have been able to conduct the BMP4 analysis without the bench work and efforts of everyone in the DeAngelis Lab, including Rosann, Denise, and Catrina. The genetic statistics were run by Margaux Morrison, without whom many last minute detail questions would have remained unanswered.

Thank you to David Hunt and the entire Smithsonian National Museum of Natural History for allowing my access to the Terry Collection for my research. This research at NMNH was funded in part by the University of Utah Department of Anthropology.

Finally, I would like to thank my parents, Dr. Patrice Dardenne, and Roxanne Dardenne for their unwavering support of my interests, and for being my sounding board, my editors, and for always answering those 3am phone calls. I love you both very much.

INTRODUCTION

The Need for More Avenues of Identification

Forensic anthropology, especially osteological analysis, has become vital to medico-legal cases in which remains are highly desiccated or void of soft tissue. One of the main roles of the anthropologist in these cases is the attempted identification of the deceased through the implementation of identification methodologies and the creation of a biological profile of the remains. Unfortunately, there are only a few databases to which researchers can compare their findings to help identify the deceased. Without the ability to compare the biological profiles of unidentified remains to potential matches, it is very difficult to give a name to the unknown remains. At the same time, there are many medical patient databases with extensive areas of information that have yet to be utilized by outside sources, especially forensic investigators. The creation and exploration of innovative approaches to identification in forensic anthropology is vital to both the growth of the field and future attempts to name the unidentified in medico-legal situations. The aim of this research is to demonstrate that collaboration between genetic and forensic researchers can expand skeletal identification techniques and provide new avenues for individual identification through the use of specific medical databases and unique skeletal diseases.

Age-Related Macular Degeneration

Utilizing disease-specific databases may provide a much needed tool for forensic anthropologists in their work to identify the unknown. By using a medical database associated with the eye disease Age-Related Macular Degeneration (AMD), forensic anthropologists have the chance to improve their identification and individualization process. AMD is an inflammatory, late-onset, progressive, degenerative disease that primarily affects the macula, or central region, of the human retina - the light-sensitive tissue lining the inner part of the eye – and is the leading cause of blindness in the US (DeAngelis et al., 2004; Swaroop et al., 2009; Klein et al., 2011). AMD causes minor to advanced central vision loss in individuals over 50 years and currently affects over 20 million people worldwide, with 1.75 million US citizens having advanced forms of the disease in at least one eye (Friedman et al., 2004; EDPRG, 2011). In its early stages it causes only minimal visual impairment, but may lead to loss of high-resolution central vision in severe cases, which in turn leads to loss of the ability to drive, recognize faces, and read. As the population ages, AMD will be a more common cause of vision loss than diabetic eye disease and glaucoma combined, one of the primary reasons being that the prevalence of AMD increases dramatically with age.

Early stages of the disease are characterized by yellowish deposits in the macula and peripheral retina, known as drusen, and generalized retinal pigment epithelium (RPE) changes (Jager et al., 2008). Drusen can be small (under 63μm), medium (63-124μm), or large (over 124μm), and have been clinically classified as hard, soft, cuticular, calcified, and most recently, reticular (Jager et al, 2008; Schmitz-Valckenberg et al., 2011). The presence of a few medium-sized drusen is the hallmark sign of early AMD, however,

there are two recognized forms of advanced AMD which do not solely rely on the presence of drusen for characterization. Dry AMD is the less severe form and accounts for 90% of cases, and wet AMD is the more severe form and only accounts for 10% of cases (DeAngelis et al., 2011). Dry AMD results in geographic atrophy, which is the disruption of the natural geography of the retina associated with a slow, progressive deterioration of the RPE, resulting in the loss of photoreceptors in the localized area (Swaroop et al., 2007). Wet AMD is associated with the neovascular presentation of the disease, which is the creation of new, and secretion of existing blood vessels (Swaroop et al., 2007; Feehan et al., 2011). The progression of AMD is classified into four categories (Figure 1) known as the AREDS system (Davis et al., 2005). The categories range from

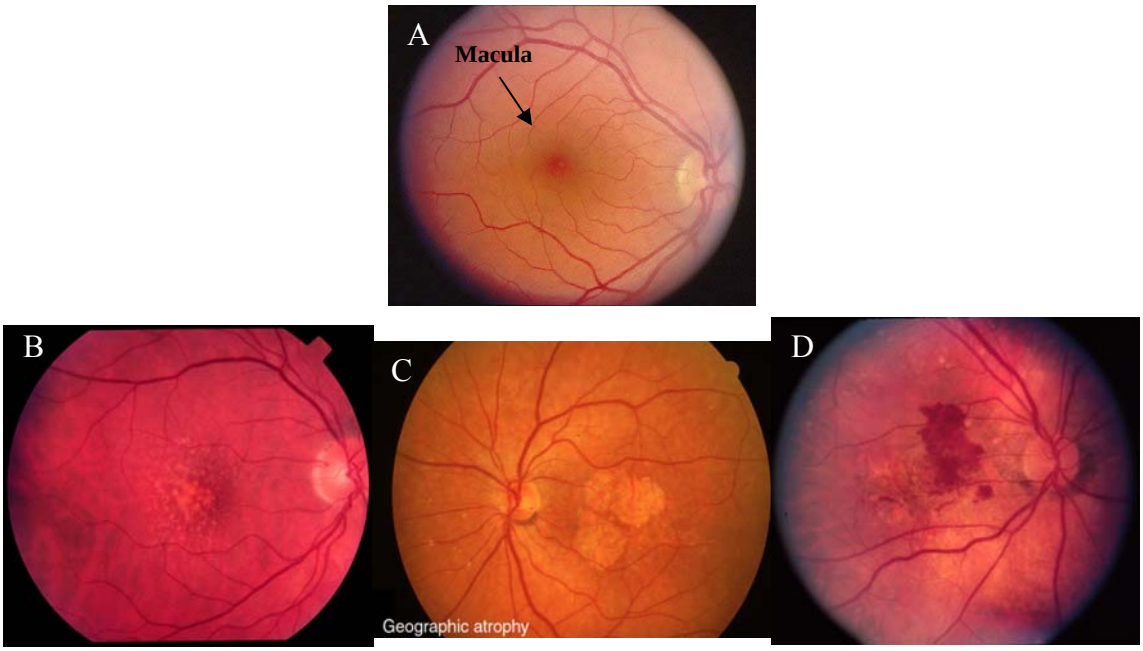

Figure 1. The AREDS stages of AMD. Clinical fundus photo of a right human retina (A) with the yellow macular region identified. B is the early to intermediate stages of AMD classified as categories 2-3 on the AREDS scale, with drusen present. In C there is evidence of advanced dry AMD in the form of geographic atrophy; an AREDS level 4. D shows advanced, neovascular, wet AMD as characterized by the red mass near the macula which is a secretion from the blood vessels in the retina, also an AREDS level 4.

no signs of disease to advanced AMD and are characterized by increased abnormalities present at and around the macula.

Extensive research into the etiology of AMD has indicated that there are both epidemiologic and genetic risk factors to the disease. The most important environmental factor is smoking, with individuals smoking 10 pack-years or more having a 144-fold increased risk of developing AMD over those who have not smoked to such a degree (DeAngelis et al., 2007). Other significant risk factors include: age, body mass index, dietary fat intake (especially lard and solid fats), hypertension, low dietary intake of antioxidants and zinc, serum cholesterol/use of cholesterol lowering medications, alcohol consumption, diabetes mellitus, sunlight exposure, iris color, and family history (Klein et al., 2004; Choi et al., 2011; DeAngelis et al., 2011; Ngai et al., 2011). Research has also revealed factors which are protective against the development of AMD. Evidence has suggested that consumption of omega-3 fatty acids in association with antioxidants, as well as Vitamin D intake may be protective against AMD development and neovascular AMD, respectively (Kishan et al., 2011; Morrison et al., 2011).

Genetic studies have identified Compliment Factor H (CFH) as a major risk gene located on chromosome 1 (Klein et al., 2005; Haines et al., 2005; Edwards et al., 2005). Further analysis has shown an even stronger additive genetic component with the influence of Age-Related Maculopathy Susceptibility 2 /HTRA Serine Peptidase 1 (ARMS2/HTRA1) on chromosome 10 (Yang et al., 2006; DeAngelis et al., 2008; Andreoli et al., 2009). Specifically, the ARMS2 risk allele is associated with earlier and more severe neovascular AMD (Leveziel et al., 2010). In fact, the chromosome 10 region where HTRA1 resides is thought to have the strongest association with risk of

neovascular AMD among any of the currently accepted AMD genetic risk factors (AMD Gene Consortium, 2013). CFH is thought to be most influential in the onset of AMD, while the combination of CFH and ARMS2/HTRA1 account for the advancement of AMD through the AREDS stages (Zhang et al., 2008; Farwick et al., 2009). In addition, the genes ROBO1 and RORA have also been shown to interact with both CFH and ARMS2/HTRA1 and significantly increase the risk of developing AMD (Schaumberg et al., 2010; Jun et al., 2011). Overall, the genetic evidence strongly suggests the role of many genes and genetic factors in the susceptibility if individuals to both forms of AMD (Sturgill et al., 2006; Kim et al., 2008; DeAngelis et al., 2011; Hong et al., 2011).

The earliest suggestion of genetic predisposition to the disease was identified through familial aggregation studies that showed AMD clusters in families, i.e., patients with AMD are more likely to have relatives with AMD (Silvestri et al., 1994; Seddon et al., 1997). While age is the biggest risk factor for developing AMD, an average person is 3-6 times more likely to have AMD if he/she has an affected parent or sibling. Later twin studies showed that AMD concordance is greater for identical twins than fraternal twins (Swaroop et al., 2009; DeAngelis et al., 2011). Studies also showed that White individuals are at higher risk of developing the disease than any other ethnic group (Klein et al., 2006; Klein et al., 2011). These early genetic studies prompted a search of the genome for genes that are working differentially between the different types of AMD and between those with the disease and those without, even among siblings. A remarkable convergence of clinical, biological, and genetic data has made AMD one of the best characterized complex trait diseases.

Connecting Bone and Eye Disease

Recent research on the genetic pathways related to AMD has identified the gene Bone Morphogenetic Protein 4 (BMP4) as being associated with previously identified major risk factors of the disease (Zhu et al., 2009a; Zhu et al., 2009b; Silveira et al., 2010; Xu et al., 2011). This gene plays an important role in the onset of endochondral bone formation and fracture repair in humans. Alterations in expression of the gene, as well as specific variants found within the gene, have been associated with a variety of bone diseases. Specifically, the single-nucleotide polymorphism (SNP), or DNA sequence variation of a single base, rs17563 in BMP4 has been associated with the bone disease ossification of the posterior longitudinal ligament (OPLL) (Meng et al., 2010). OPLL occurs when the ligament running along the vertebral bodies within the spinal column starts to ossify, or become bone, and impinge on the spinal cord potentially causing neurological complications (Singh et al., 2004).

The association of both an identifiable skeletal abnormality and a well documented eye disease with the same risk gene makes OPLL a prime candidate for an avenue of forensic identification through a combined physical and genetic methodology. With approximately 10% of the population over 40 years, and 30% of the population over 75 years presenting with some form of the disease, it is projected that about 2.95 million Americans will have advanced AMD by the year 2020 (Klein et al., 1992; Friedman et al., 2004; EDPRG, 2011). As a result, there is a rich knowledge base on the individuals who suffer from this disease throughout the country. Because of the disease's association with BMP4, forensic anthropologists have the chance to utilize this wealth of data linked to AMD (i.e., medical records) to facilitate the identification of human remains.

Format of Research

The intent of this research is to show that, given the genetic association between AMD and OPLL via BMP4, the identification of OPLL on a set of remains can allow the forensic anthropologist to direct a DNA study in search of the SNP in the BMP4 gene related to AMD. Upon finding the SNP, analysis would continue in search of the other known risk SNPs which interact with BMP4, and suggest that the individual does have AMD. The anthropologist can then search the local AMD patient databases, using the biological profile, to advance the identification process by composing a subset of potential matches.

This research is intended to be applicable for use by forensic anthropologists in the United States, as a result, it focuses on the two largest population groups in the US according to the 2010 census which comprise 85% of the total population, Whites and Blacks (Humes et al., 2011). Primary interest rests with individuals of European ancestry (referred to as "Whites" for the remainder of the paper) as they represent the majority of individuals in the United States (72.4%), they are the highest at-risk group for development of AMD (Klein et al., 2011), and they are believed to be the population with the lowest prevalence of OPLL (Matsunaga and Sakou, 2006b). The high prevalence of AMD among Whites provides a great deal of information on those with the disease, while the proposed low prevalence of OPLL means that this identification method will actually be useful in narrowing down the pool of potential matches from the wider population. The addition of Black individuals in the skeletal study, as well as an Asian population in the genetic analysis, provides the chance for a comparative study and the observation of any differences between populations.

The research presented here was conducted in two main avenues of analysis. First, a genetic analysis was conducted with the BMP4 gene in relation to AMD among four separate cohorts. Variation in the gene was analyzed for direct risk of AMD and for its interaction with other known risk factors of the disease. This was done to confirm that BMP4 is in fact functioning in the pathogenesis of AMD and that the SNP associated with OPLL, rs17563, was the risk factor tying these two diseases together. Secondly, OPLL has only been extensively studied in Asians, in a clinical setting. To better understand the prevalence and characteristics of this disease in America, analysis was conducted on a 20th Century skeletal population of White and Black individuals. This analysis was able to clarify the utility of the proposed identification method among the majority of the US population. The following chapters will introduce the two main research aspects of this work, BMP4 and OPLL, and present the results of each analysis. The OPLL-BMP4-AMD Identification Method section will discuss how these results affect the applicability of the proposed method and where to direct future research. The Conclusion shall present the final analysis of the original hypothesis and discuss what this research has brought to the field of anthropology, beyond its original intent.

Improving Identification Through Collaboration

There are currently over 40,000 unidentified remains in the United States (Ritter, 2007); carrying out innovative research such as this can help identify these remains and bring closure to the families of the missing. This project is an original exploration of the use of disease-specific medical databases for forensic identification. The research integrates traditional forensic anthropological identification techniques with the use of

disease-specific patient genetic databases to create a novel avenue of forensic identification. This work can advance the knowledge base of forensic anthropologists by suggesting that features not previously considered individuating, such as OPLL, can in fact become vital parts of the identification process with a little background research and collaboration with other scientists. This research also has the potential to develop better communication and understanding between forensic anthropologists and medical researchers.

BONE MORPHOGENETIC PROTEIN 4

Introduction

As was mentioned in the Introduction section of this paper, this research is a result of inquiry into the genetic network of AMD and the identification of BMP4 as a risk allele for this important disease. Recent research on the genetic pathways related to AMD has identified BMP4 in the gene network related to the RORA-ROBO1-HTRA1 triumvirate, which conveys the highest collective risk for AMD (Silveira et al., 2010). This RORA-ROBO1-HTRA1 network is a visualization of the connection between the genes implicated in AMD, as identified through previous research and published through the National Center for Biotechnology Information (NCBI). This pathway (Figure 2) presents genes which have been identified numerous times as having an important association with, and are generally accepted to increase the risk of AMD. It also begins to explain the connections between the numerous candidate genes. BMP4 is identified as having a suspected direct association with HTRA1. This is the first method of inquiry into the pathogenesis of AMD which identified BMP4 as a potential gene of interest (Silveira et al., 2010). The presence of BMP4 in this network suggests that it plays an important role in connection with the RORA-ROBO1-HTRA1 triumvirate as vital to the pathogenesis of AMD. If that is truly the case, identification of variants within this gene that are direct risk factors for AMD and that are associated with OPLL, such as SNP rs17563 (or in linkage with this SNP) will help establish the genetic connection between

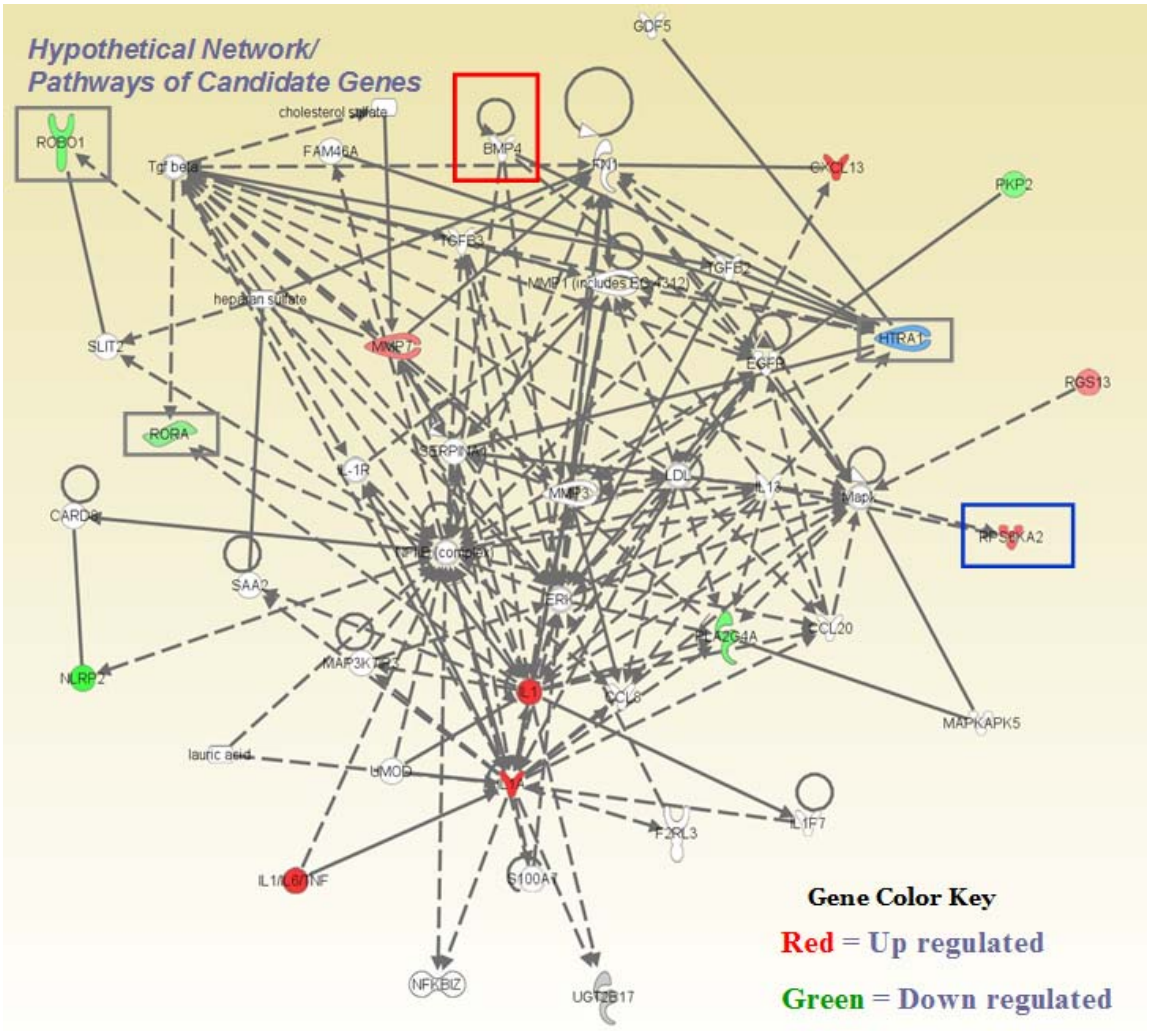

Figure 2. The RORA-ROBO1-HTRA1 network. This network is constructed from the available data on candidate genes associated with the eye disease AMD. The genes within the grey boxes have been identified numerous times as having an important association with, and are generally accepted to increase the risk of AMD. Connections between genes identified with hash marks represent un-tested or tenuous associations. Solid lines represent direct, or suspected direct interaction between genes. You can see BMP4 identified in the red box as having a suspected direct association with HTRA1 when discussing AMD. Modified from Silveira et al, 2010.

these two diseases and provide the basis for the proposed method of identification.

Explanation of the BMP4 Gene

The BMP4 gene identified through this network is a regulatory molecule that operates throughout development in mesoderm induction, tooth development, limb formation, bone induction, and fracture repair (Kochanowska et al., 2007; Bakrania et al., 2008; Pachori et al., 2010). BMP4 is a member of the bone morphogenetic protein (BMP) family which originally derived its name from the proteins' ability to induce bone and cartilage formation (Urist, 1965; Wozney et al., 1988). However, BMPs are expressed in a variety of tissues in addition to bone, including cartilage, blood vessels, skin, heart, kidney, liver, lung, and the retina (Massague and Chen, 2000; Chen et al., 2004). In addition, BMPs exhibit a wide range of biological activities, from the formation and patterning of various tissues to the morphogenesis of a number of organs, and BMP-mediated signaling affects diverse cellular processes such as proliferation, differentiation, migration, and apoptosis (Hogan, 1996). BMPs are part of the transforming growth factor-beta (TGF-β) superfamily which consists of secretory signaling molecules responsible for controlling structure-related cell activities and play essential roles in embryonic development (Bakrania et al., 2008).

The human BMP4 gene is located on chromosome 14, at 14q22-q23 (Tabas et al., 1993). The gene contains five exons: exon 1A, exon 1B, exon 2, 3, and 4 (van den Wijngaard et al., 1996; Shore et al., 1998; Helvering et al., 2000). Exons 1A and 1B are alternative start sites for the various BMP4 variants, thus each variant only has four exons. There are three mRNA variants for this gene and they all encode the same

protein, derived from the sequences of only exon 3 and exon 4. Variants 1 (Figure 3) and 2 are derived from promoter A, located upstream of exon 1A, and variant 3 is derived from promoter B, located upstream of exon 1B (van den Wijngaard et al., 1999; van den Wijngaard et al., 2000; Thompson et al., 2003). Evidence indicates that there has been strong conservation of variation of this gene within species: 95% (+) identity among all of the disease SNPs within members of the primate family, and 75% (+) identity among a number of mammals varying from livestock (cattle, sheep, etc.) to elephants (Dardenne, 2012). In addition, the human BMP4 gene and mouse BMP4 gene share a similar structure and the coding regions are highly homologous, providing for very informative murine studies of the gene (Shore et al., 1998).

BMP4 and the Eye

BMP4 is particularly important in ocular development, although research has reported the expression of numerous BMPs (BMP2, BMP3, BMP4, BMP5, BMP6, and BMP7) in ocular tissues (Wordinger and Clark, 2007). In chick models, BMP4 signaling has been found to control the size of the dorsal optic cup by regulating cell proliferation and apoptosis, and in general, BMPs have been shown to be necessary, and sufficient, for RPE development *in vivo* (Behesti et al., 2006; Muller et al., 2007). In murine models, lens induction is absent in mice with homozygous disruption of BMP4, and $BMP4^{+/-}$ heterozygotes develop a variety of ocular abnormalities including elevated intraocular pressure, anterior segment dysgenesis, and posterior segment abnormalities (Furuta and Hogan, 1998; Chang et al., 2001). Indirect suppression of BMP4 can result in optic nerve

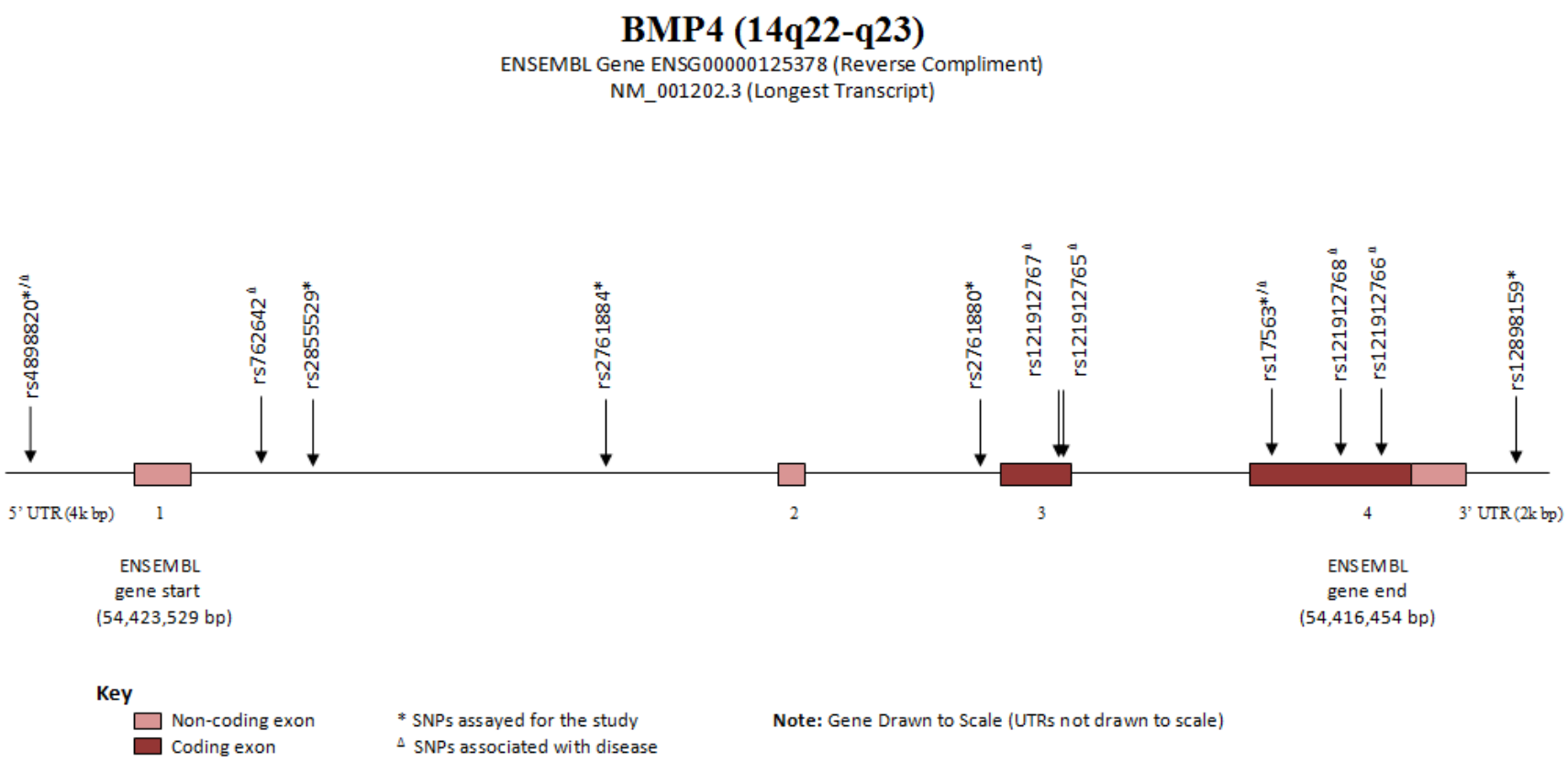

Figure 3: BMP4 gene schematic. Representation of the longest variant of the BMP4 gene constructed from the available literature. The location, direction, and exons of the gene are visible, as well as the SNPs that were assayed for this research and the SNPs associated with diseases described below.

aplasia and microphthalmia in Msx2 transgenic mice (Wu et al., 2003). In rats, BMP4 has been shown to mediate apoptosis of capillary endothelial cells during pupilary membrane regression (Kiyono and Shibuya, 2003). However, BMP4 has also been associated with positive effects in the eye, including short-lived protective effects on retinal neurons from NMDA-mediated excitotoxicity, suppression of damage-induced proliferation of Muller glia, and the inhibition of exaggerated RPE proliferation in wound repair (Mathura et al., 2000; Fischer et al., 2004).

In regards to eye disease, BMP4 has long been implicated in the pathogenesis of glaucoma, especially in regards to elevated intraocular pressure, and it is a strong candidate gene for Axenfeld-Rieger Anomaly and other conditions associated with glaucoma (Chang et al., 2001; Wordinger et al., 2007). Recently, studies out of the University of Southern California indicated that BMP4 may be involved in the determination of which late form of AMD an individual develops. This team of researchers demonstrated that BMP4 was differentially expressed in the macular tissues of "dry" and "wet" AMD patients (Zhu et al., 2009a; Zhu et al., 2009b; Xu et al., 2011). In the RPE and choroid tissues of the macula in late dry AMD, BMP4 expression was increased. Oxidative stress increased BMP4 expression in these RPE cells and BMP4 acted as a mediator in oxidative stress-induced RPE senescence (Zhu et al., 2009a). In contrast to the up-regulation of BMP4 in dry AMD, choroidial neovascular (CNV), or wet, AMD is characterized by a low expression of BMP4 in the RPE cells (Xu et al., 2011). Murine models show that BMP4 expression is decreased in laser-induced CNV mice when tumor necrosis factor (TNF) levels are high, and rebounds when TNF levels are low (Xu et al., 2011). Thus, BMP4 may be involved in the molecular switch

determining which phenotypic pathway is taken in the progression from early to late stage AMD.

BMP4 and Human Disease

There are a number of diseases outside the eye which are also associated with this gene, such as HFE hemochromatosis penetrance, scoliosis, cleft palate with or without cleft lip, oligodontia, and juvenile leukemia, among others (Figure 4) (Milet et al., 2007; Suzuki et al., 2009; Simón-Sánchez et al., 2009; Zhang et al., 2009; Hi et al., 2010; Suazo et al., 2010; Mórocz et al., 2011; Olk-Batz et al., 2011; Mu et al., 2012). In general, mutations in BMP4 are known to cause eye, brain, and digit abnormalities (Bakrania et al., 2008). BMP4 can also act as an antiangiogenic factor by inducing apoptosis (Fujita et al., 1999; Kiyono and Shibuya, 2003; Fukuda et al., 2006; Kiyono and Shibuya, 2006) and cell senescence (Buckley et al., 2004; Su et al., 2009; Zhu et al., 2009a). BMP4 has been shown to be over expressed in a number of diseases including lumbar and hindlimb fusion, hypoxia-induced pulmonary hypertension, colorectal cancer, and fibrodysplasia ossificans progressiva, which is a genetic disorder of axial and appendicular skeletal malformation and progressive heterotopic ossification (Shafritz et al., 1996; Shore et al., 1998; Blaszczyk et al., 2003; Frank et al., 2005; Feldman et al., 2007; Houlston et al., 2008; Fernandez-Rozadilla et al., 2010; Lubbe et al., 2010; Niittymaki et al., 2011; Tang et al., 2011; Kaplan et al., 2012; Kaplan et al., 2012; Lubbe at al., 2012; Suzuki et al., 2012; Fernandez-Rozadilla et al., 2013). BMP4 has been implicated in ovarian cancer specifically, and as a potential therapeutic target in cancer cell research in general, and a strong expression of BMP4 in malignant melanomas has been shown to promote cell

BMP4 and Human Disease

Disease	Variation	Location	Source
Adolescent idiopathic scoliosis	rs4898820	promoter	Mórocz et al. 2011
AMD	Over/under expression		Zhu et al. 2009a,b; Xu et al. 2011
Axenfeld-rieger anomaly (glaucoma)	Underexpression		Chang et al., 2001
Colorectal Cancer	rs4444235: p.W325C, p.C373S, p.I381V	Downstream of exon 4	Houlston et al., 2008; Lubbe et al., 2010; Niittymaki et al., 2011; Lubbe et al., 2012; Fernandez-Rozadilla et al., 2013
Cutaneous melanoma	rs17563	exon 4	Capasso et al., 2009
Fibrodysplasia ossificans progressiva	Overexpression		Shafritz et al., 1996; Blaszczyk et al., 2003
HFE Hemochromatosis Penetrance	rs4901474	Upstream of exon 1	Milet et al., 2007
Juvenile myelomonocytic leukemia	Hypermethalation		Olk-Batz et al., 2011
Microphthalmia Syndromic type 6	rs121912765	exon 3	Bakrania et al., 2008
Nonsyndromic cleft lip with or without cleft palate	rs762642	intron 1	Suazo et al., 2010
Oligodontia	c.455T>C	exon 4	Mu et al., 2012
Orofacial Cleft 11	rs121912766; rs121912767; rs121912768	exon 4; exon 3; exon 4	Suzuki et al., 2009
Ossification of the posterior longitudinal ligament	rs17563	exon 4	Meng et al., 2010
Otosclerosis	rs17563	exon 4	Schrauwen et al., 2008

Figure 4. BMP4 and human disease. Diseases associated with BMP4 variants and dysregulation of gene expression. See Figure 3 for precise location of SNPs within the gene itself.

invasion and migration (Kochanowska et al., 2002; Shepherd and Nachtigal, 2003; Rothhammer et al., 2005; Rothhammer et al., 2007; Theriault et al., 2007; Shepherd et al., 2008; Kallioniemi et al., 2012; Peart et al., 2012). There is strong evidence that individuals with a BMP4 disease risk allele also have, or are more susceptible to, other diseases associated with the gene (such as those described above).

One BMP4 SNP in particular, rs17563, is associated with a number of diseases including cutaneous melanoma, otosclerosis (abnormal boney growth near the middle ear), and ossification of the posterior longitudinal ligament of the cervical spine (OPLL)

(Figure 4) (Schrauwen et al., 2008; Capasso et al., 2009; Meng et al., 2010; Ren et al., 2012a). Focusing on OPLL, it has been shown that the T allele in rs17563 among Han Chinese males increases the risk of development of OPLL and causes more extensive OPLL throughout the cervical spine (Meng et al., 2010; Ren et al., 2012a). More generally, BMP4 has been identified as a risk gene through large-scale screenings of various populations, suggesting that there is a ubiquitous OPLL risk factor in this gene across populations (Furushima et al., 2002; Tanno et al., 2003). OPLL is the focus of this research because of its identifiable, physical manifestation in the skeleton, and rs17563 is a prime target for the connection between OPLL and AMD because of its previously reported multidisease association.

Genetic and Interaction Analyses

Introduction

While the previous research highlighting the involvement of BMP4 in the advancement of AMD is encouraging for this research, the identification method proposed here requires more than just dysregulation of gene expression as a risk factor. In order for BMP4 to be a target of identification, the risk factor for AMD has to be the identifiable variant, rs17563, which is directly associated with risk of OPLL, or in linkage with rs17563. Linkage, or linkage disequilibrium (LD), refers to certain combinations of alleles being inherited together more often than would be expected by random chance. Thus, this method is applicable whether the two diseases share a common risk allele or if the risk alleles are in LD (therefore more likely to be seen and inherited together). However, in order to exhaust all possibilities of BMP4 involvement in AMD, a number

of SNPs were selected to capture the full variation within the gene to test for direct association between specific BMP4 loci and risk of AMD, as well as, interaction between BMP4 SNPs and previously reported AMD-risk SNPs within the RORA-ROBO1-HTRA1 triumvirate.

Patient Populations

This study was comprised of four cohorts, two sibling cohorts and two unrelated cohorts. A subset of the New England Sibling Cohort (NESC) (n=418), and an additional sibling cohort provided by Giuliana Silvestri (GSSibs) from Belfast, Northern Ireland (n=119) comprise the related study groups. Two additional cohorts consisting of 436 unrelated individuals from central Greece (Greeks) and 1333 unrelated individuals from Korea (Koreans) were also studied. The two sibling cohorts and the Greek cohort were comprised entirely of White individuals. Details of recruitment, diagnostic criteria and subject classification for the NESC are described elsewhere (DeAngelis et al., 2007; Silveira et al., 2010). But in brief, at least one individual from each family had the neovascular (wet) form of AMD in at least one eye after excluding patients with a retinal pigment epithelium detachment, myopia, ocular histoplasmosis syndrome, angioid streaks, choroidal rupture, any hereditary retinal diseases other than AMD, and previous laser treatment for retinal conditions other than AMD. All but 87 sibling pairs in the original NESC cohort were discordant for AMD. The Greek cohort was enrolled at the University Hospital of Larissa outpatient medical clinics in central Greece. The diagnosis of AMD in this cohort was confirmed by optical coherence tomography and fluorescent angiography (DeAngelis et al., 2007; Silveira et al., 2010).

Both the GSSibs and Koreans are newly composed patient populations and recent additions to the DeAngelis Lab's collection of cohorts. As such, the collaborators from Ireland and South Korea have yet to provide detailed reports on their patient participation protocol. Both cohorts were comprised of individuals being treated for AMD at registered medical clinics, and due to commonly accepted practices in AMD research, the inclusion of individuals into the study populations would be similar to those described above.

Methods

Initially, four BMP4 SNPs (rs12898159, rs2761880, rs2761884, and rs2855529) were genotyped on two cohorts: a subset of the NESC sibling cohort (n=418) and the Greek cohort (n=436). Linkage disequilibrium between the genotyped SNPs was determined using Haploview. Linkage disequilibrium among the SNPs was similar between disease subtypes (not shown) and is shown for the NESC cohort in Figure 5.

SNPs rs2761884 and rs2855529 were shown to be in complete LD, but the rest of the SNPs were not in high LD ($r^2<0.8$). After the primary SNP selection, the tagging SNP rs17563 (which is also a coding SNP) and the SNP rs4898820, which represents the variation from the second LD block in ABI SNPBrowser, were genotyped to cover the remainder of the variation within the BMP4 gene.

All SNPs were tested for their association with AMD and its subtypes using both conditional logistic regression (CLR) and generalized estimating equations (GEE) for the related individuals, and logistic regression for the unrelated cohorts in SAS, testing each genetic model. SNPs showing evidence of association in the preliminary analysis were

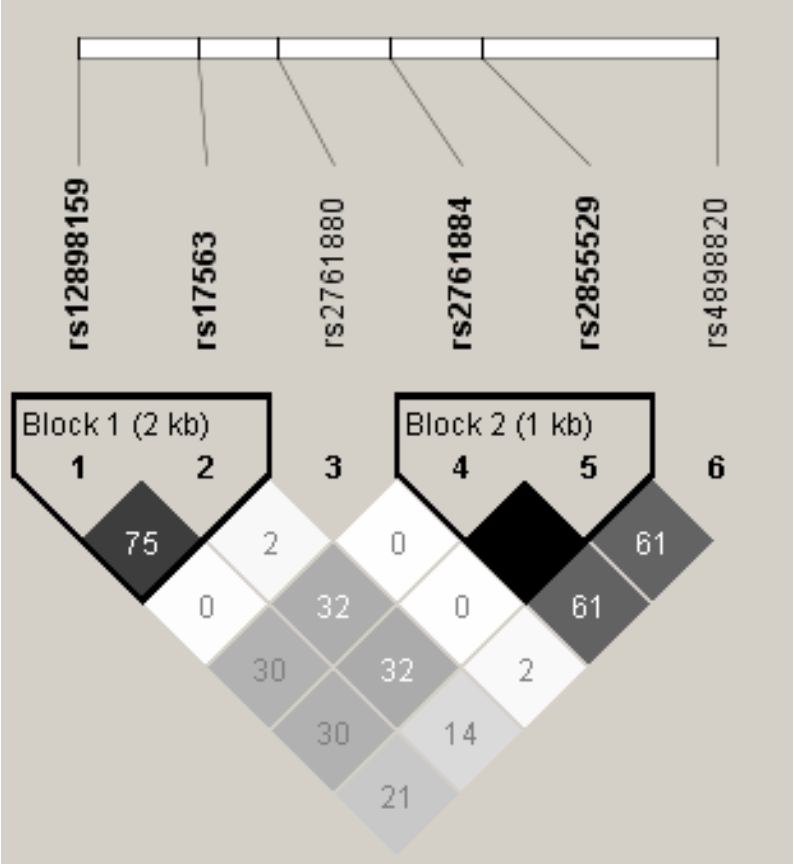

Figure 5. Linkage Disequalibrium (LD) among the NESC sibling cohort.

then genotyped in the unrelated Korean cohort and the additional sibling cohort (GSSibs). Meta-analysis of the cohorts was performed using Comprehensive Meta-Analysis v2. Adjustments were made for the effects of age and sex so that the model tested the effect of the SNP if everyone in the cohort was the same age and sex. Tests for interaction between BMP4 SNPs and previously associated SNPs in ARMS2/HTRA1, RORA, and ROBO1 were performed by adding an interaction term into the regression model. Additionally, a cases-only interaction analysis was performed using regression. SNPs showing association at $p \leq 0.2$ in any cohort were tested for interaction with SNPs from genes in the RORA-ROBO1-HTRA1 pathway that included BMP4.

Results

Of the first 4 SNPs genotyped, one SNP, rs12898159, showed significant association with both "all AMD subtypes" and neovascular AMD in the subset of the NESC cohort (p = 0.0084 and 0.0142, respectively; Table 1a). No BMP4 SNPs showed significance in the Greek cohort (Table 1b). Analysis of all the SNPs in the four cohorts did not show any SNPs significant among all cohorts (Tables 2-4). Additionally, the two methods of analysis in the family cohorts (GEE and CLR) did not show consistent results, i.e., rs12898159 was shown to be highly significant in neovascular vs. normal in the NESC by GEE (p = 0.00E-00) but was not significant by CLR (p = 0.1401). The complete results from all analyses can be found in Appendix A.

The most significant SNP in the NESC was rs12898159 by GEE, which was shown to increase risk of neovascular AMD under an additive model (p = 0.00E-00), and rs17563 by CLR, which was shown to increase risk of all AMD subtypes under a recessive model (p =.01). The most significant SNP in the Greeks was rs2761884, which was shown to be protective against neovascular AMD under a recessive genetic model (p = 0.03). The most significant SNP in the GSSibs by GEE was rs4898820, which was shown to decrease risk of all AMD subtypes under a dominant model (p = 0.03), and the CLR analysis showed no significant associations for this cohort. In the Korean cohort, the most significant SNP was rs17563, which was shown to increase risk of dry AMD under a recessive model (p = 0.0097).

Combining all cohorts, and using the cases-only interaction analysis, a more powerful measure of interaction, significant interaction was seen between the BMP4 SNP rs17563 and SNPs in both ROBO1 and RORA. Specifically, after correction for multiple

Table 1: Single SNP Analysis for BMP4

1a. NESC*		Neo v Normal				All AMD v Normal			
SNP	Allele	Odds ratio	95% CI low	95% CI high	p value	Odds ratio	95% CI low	95% CI high	p value
rs2761880	A	0.333	0.035	3.205	0.3414	0.333	0.035	3.205	0.3414
rs2761884	T	0.646	0.379	1.102	0.1092	0.685	0.408	1.151	0.1529
rs2855529	T	0.686	0.402	1.171	0.1671	0.726	0.431	1.222	0.2284
rs12898159	A	2.108	1.162	3.826	0.0142	2.172	1.22	3.869	0.0084

1b. Greeks*		Neo v Normal				All AMD v Normal			
SNP	Allele	Odds ratio	95% CI low	95% CI high	p value	Odds ratio	95% CI low	95% CI high	p value
rs2761880	A	0.796	0.428	1.483	0.4726	0.987	0.587	1.660	0.9600
rs2761884	T	0.872	0.636	1.195	0.3941	0.954	0.722	1.260	0.7401
rs2855529	T	0.875	0.637	1.203	0.4116	0.953	0.721	1.260	0.7353
rs12898159	A	1.201	0.874	1.65	0.2592	1.034	0.779	1.372	0.8162

*The single SNP analysis of the first four BMP4 SNPs in the (1a) NESC Sibling Cohort and the (1b) Greek Cohort.

Table 2: BMP4 SNP Analysis on All AMD Subtypes

Model	Study name	Subgroup within study	Comparison*	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value
	Greeks	AllAMD	rs1884dom	1.147	0.775	1.698	0.685	0.4931
	NESC	AllAMD (CLR)	rs1884dom	0.293	0.089	0.965	-2.018	**0.0436**
Fixed				1.004	0.692	1.457	0.021	0.9836
	Greeks	AllAMD	rs7563add	1.063	0.802	1.408	0.426	0.6705
	Koreans	AllAMD	rs7563add	1.098	0.909	1.326	0.971	0.3317
	GSSibs	AllAMD (CLR)	rs7563add	0.644	0.192	2.161	-0.713	0.4761
	NESC	AllAMD (CLR)	rs7563add	1.704	1.037	2.801	2.103	**0.0355**
Fixed				1.123	0.968	1.302	1.528	0.1266
	Greeks	AllAMD	rs7563rec	0.978	0.626	1.527	-0.098	0.9221
	Koreans	AllAMD	rs7563rec	1.113	0.88	1.408	0.892	0.3726
	GSSibs	AllAMD (CLR)	rs7563rec	0.438	0.05	3.82	-0.747	0.455
	NESC	AllAMD (CLR)	rs7563rec	2.762	1.207	6.319	2.406	**0.0161**
Fixed				1.135	0.928	1.387	1.232	0.2178
	Greeks	AllAMD	rs8820dom	1.104	0.708	1.721	0.437	0.6624
	Koreans	AllAMD	rs8820dom	1.148	0.89	1.481	1.062	0.288
	GSSibs	AllAMD (GEE)	rs8820dom	0.335	0.118	0.947	-2.063	**0.0391**
	NESC	AllAMD (GEE)	rs8820dom	0.929	0.643	1.341	-0.394	0.6936
Fixed				1.038	0.862	1.25	0.391	0.6958

* Results of meta-analysis were conducted under three different genetic models (additive, dominant, and recessive) for all AMD subtypes, after adjustment for age and sex. Only the significant analyses are presented (colored in pink).

Table 3: BMP4 SNP Analysis on Neovascular AMD

Model	Study name	Subgroup within study	Comparison*	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value
	Greeks	NeoNorm	rs8159add	1.171	0.848	1.618	0.958	0.3382
	GSSibs	NeoNorm (GEE)	rs8159add	0.996	0.532	1.866	-0.011	0.991
	NESC	NeoNorm (GEE)	rs8159add	2.488	2.144	2.886	12.036	**0**
Fixed				2.107	1.847	2.404	11.081	**0**
	Greeks	NeoNorm	rs8159dom	1.219	0.748	1.987	0.795	0.4269
	GSSibs	NeoNorm (GEE)	rs8159dom	0.963	0.432	2.151	-0.091	0.9277
	NESC	NeoNorm (GEE)	rs8159dom	1.686	1.036	2.744	2.102	**0.0355**
Fixed				1.348	0.982	1.851	1.848	0.0646
	Greeks	NeoNorm	rs8159rec	1.241	0.708	2.175	0.754	0.4506
	GSSibs	NeoNorm (GEE)	rs8159rec	1.069	0.26	4.4	0.093	0.9259
	NESC	NeoNorm (GEE)	rs8159rec	2.053	1.035	4.071	2.059	**0.0395**
Fixed				1.474	0.973	2.232	1.833	0.0669
	Greeks	NeoNorm	rs8820add	0.903	0.667	1.223	-0.659	0.51
	Koreans	NeoNorm	rs8820add	0.922	0.761	1.117	-0.831	0.4057
	GSSibs	NeoNorm (GEE)	rs8820add	0.654	0.347	1.232	-1.315	0.1886
	NESC	NeoNorm (GEE)	rs8820add	1.623	1.127	2.337	2.605	**0.0092**
Fixed				0.985	0.853	1.137	-0.208	0.8351
	Greeks	NeoNorm	rs8820dom	1.012	0.612	1.673	0.046	0.9629
	Koreans	NeoNorm	rs8820dom	1.14	0.845	1.537	0.859	0.3906
	GSSibs	NeoNorm (GEE)	rs8820dom	0.335	0.118	0.947	-2.063	**0.0391**
	NESC	NeoNorm (GEE)	rs8820dom	1.944	1.042	3.625	2.091	**0.0366**
Fixed				1.126	0.893	1.42	1.004	0.3153

* Results of meta-analysis were conducted under three different genetic models (additive, dominant, and recessive) for neovascular AMD, after adjustment for age and sex. Only the significant analyses are presented (colored in pink).

Table 3: Cont.

Model	Study name	Subgroup within study	Comparison*	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value
	Greeks	NeoNorm	rs1884dom	1.05	0.672	1.64	0.214	0.8303
	NESC	NeoNorm (CLR)	rs1884dom	0.195	0.046	0.822	-2.227	**0.0259**
Fixed				0.906	0.592	1.387	-0.455	0.6491
	Greeks	NeoNorm	rs1884rec	0.465	0.23	0.94	-2.132	**0.033**
	NESC	NeoNorm (CLR)	rs1884rec	1.199	0.379	3.794	0.309	0.7575
Fixed				0.602	0.33	1.097	-1.658	0.0973
	Greeks	NeoNorm	rs8820rec	0.748	0.45	1.243	-1.121	0.2623
	Koreans	NeoNorm	rs8820rec	0.645	0.452	0.92	-2.419	**0.0156**
	GSSibs	NeoNorm (CLR)	rs8820rec	2.329	0.275	19.741	0.775	0.4382
	NESC	NeoNorm (CLR)	rs8820rec	0.824	0.378	1.795	-0.487	0.6261
Fixed				0.707	0.54	0.927	-2.51	**0.0121**
	Greeks	NeoNorm	rs1884rec	0.465	0.23	0.94	-2.132	**0.033**
	NESC	NeoNorm (GEE)	rs1884rec	1.195	0.667	2.14	0.599	0.5493
Fixed				0.814	0.52	1.275	-0.898	0.3691

* Results of meta-analysis were conducted under three different genetic models (additive, dominant, and recessive) for neovascular AMD, after adjustment for age and sex. Only the significant analyses are presented (colored in pink).

Table 3: Cont.

Model	Study name	Subgroup within study	Comparison*	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value
	Greeks	NeoNorm	rs8820rec	0.748	0.45	1.243	-1.121	0.2623
	Koreans	NeoNorm	rs8820rec	0.645	0.452	0.92	-2.419	**0.0156**
	GSSibs	NeoNorm (GEE)	rs8820rec	0.95	0.34	2.652	-0.098	0.9218
	NESC	NeoNorm (GEE)	rs8820rec	2.308	1.16	4.592	2.383	**0.0172**
Fixed				0.824	0.636	1.068	-1.465	0.143
	Greeks	NeoNorm	rs8820rec	0.748	0.45	1.243	-1.121	0.2623
	Koreans	NeoNorm	rs8820rec	0.645	0.452	0.92	-2.419	**0.0156**
	GSSibs	NeoNorm (GEE)	rs8820rec	0.95	0.34	2.652	-0.098	0.9218
	NESC	NeoNorm (GEE)	rs8820rec	2.308	1.16	4.592	2.383	**0.0172**
Fixed				0.824	0.636	1.068	-1.465	0.143

* Results of meta-analysis were conducted under three different genetic models (additive, dominant, and recessive) for neovascular AMD, after adjustment for age and sex. Only the significant analyses are presented (colored in pink).

Table 4: BMP4 SNP Analysis on Dry AMD

Model	Study name	Subgroup within study	Comparison*	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value
	Greeks	DryNorm	rs7563add	0.959	0.637	1.445	-0.2	0.8413
	Koreans	DryNorm	rs7563add	1.46	1.073	1.987	2.408	**0.0161**
	NESC	DryNorm (CLR)	rs7563add	4.204	0.668	26.452	1.53	0.126
Fixed				1.281	1.004	1.636	1.991	**0.0464**
	Greeks	DryNorm	rs7563dom	1.286	0.648	2.553	0.719	0.4721
	Koreans	DryNorm	rs7563dom	1.394	0.64	3.037	0.836	0.403
	NESC	DryNorm (CLR)	rs7563dom	0.956	0.134	6.827	-0.045	0.9642
Fixed				1.304	0.793	2.145	1.045	0.2959
	Greeks	DryNorm	rs7563rec	0.688	0.34	1.392	-1.04	0.2983
	Koreans	DryNorm	rs7563rec	1.631	1.126	2.363	2.585	**0.0097**
Fixed				1.353	0.974	1.878	1.804	0.0713
	Greeks	DryNorm	rs7563add	0.959	0.637	1.445	-0.2	0.8413
	Koreans	DryNorm	rs7563add	1.46	1.073	1.987	2.408	**0.0161**
	NESC	DryNorm (GEE)	rs7563add	1.082	0.779	1.503	0.47	0.6385
Fixed				1.189	0.977	1.449	1.726	0.0844
	Greeks	DryNorm	rs7563rec	0.688	0.34	1.392	-1.04	0.2983
	Koreans	DryNorm	rs7563rec	1.631	1.126	2.363	2.585	**0.0097**
	NESC	DryNorm (GEE)	rs7563rec	0.989	0.549	1.781	-0.037	0.9705
Fixed				1.256	0.943	1.672	1.557	0.1194

* Results of meta-analysis were conducted under three different genetic models (additive, dominant, and recessive) for dry AMD, after adjustment for age and sex. Only the significant analyses are presented (colored in pink).

testing, significant interactions were seen between BMP4 rs17563 and RORA SNPs rs730754 and rs8034864 and ROBO1 rs4513416 and rs1387665 (Table 5). The most significant interaction was between BMP4 rs17563 and RORA rs8034864 among dry AMD cases only ($p = 9.78E-09$).

Discussion

Considering these results specifically in regards to the association between BMP4 and AMD, this research has added important information to the general understanding of the underlying genetic mechanisms and interactions associated with this disease. One thing is abundantly clear: AMD is a complex and complicated disease in which risk factors and genetic interactions differ between populations, disease types, and combinations of the two. The research presented here supports the placement of the BMP4 gene within the RORA-ROBO1-HTRA1 network, as it is clearly functioning within this network as an independent risk factor for and protectorate against AMD. Moreover, SNPs within the BMP4 gene are interacting with a few specific SNPs within the RORA and ROBO1 genes to independently increase the risk of both dry and wet AMD in different situations. Not surprisingly, the BMP4 SNP rs17563 which has been tied to OPLL (Meng et al., 2010; Ren et al., 2012a) appears to be a key factor in relation to the disease; it independently increases risk for all AMD subtypes, and dry AMD specifically, among the NESC cohort and Korean cohort, respectively. In addition, this SNP is associated with the most significant interaction among genetic risk factors in both neovascular and dry AMD cases.

In looking at BMP4's direct relation to AMD, it is slightly discouraging that

Table 5: Cases-only Interaction Analysis

Cases Only: All Cohorts	**All AMD**				**Neo AMD**				**Dry AMD**			
Interaction*	Odds ratio	95% Low	95% High	p-value	Odds ratio	95% Low	95% High	p-value	Odds ratio	95% Low	95% High	p-value
RORA rs8034864 & BMP4 rs12898159	0.832	0.658	1.052	0.124	0.714	0.538	0.948	**0.0197**	1.43	0.892	2.292	0.1372
ROBO1 rs1387665 & BMP4 rs12898159	0.811	0.664	0.99	**0.0395**	0.741	0.585	0.939	**0.0133**	0.964	0.636	1.459	0.8614
ROBO1 rs4513416 & BMP4 rs12898159	1.242	1.017	1.518	**0.034**	1.409	1.11	1.789	**0.0048**	1.01	0.665	1.533	0.9634
ARMS2 rs10490924 & BMP4 rs17563	1.197	1.028	1.393	**0.0206**	1.18	0.978	1.424	0.0838	1.252	0.938	1.67	0.1268
HTRA1 rs11200638 & BMP4 rs17563	1.275	1.097	1.483	**0.0016**	1.211	1.007	1.456	**0.0422**	1.417	1.06	1.894	**0.0186**
HTRA1 rs1049331 & BMP4 rs17563	1.246	1.073	1.446	**0.004**	1.183	0.984	1.422	0.0734	1.37	1.028	1.824	**0.0314**
RORA rs730754 & BMP4 rs17563	1.32	1.136	1.533	**0.0003**	1.163	0.967	1.398	0.1086	1.928	1.432	2.596	**1.51E-05**
RORA rs12900948 & BMP4 rs17563	1.156	0.995	1.344	0.0582	1.095	0.909	1.32	0.3389	1.343	1.004	1.797	**0.0467**
RORA rs8034864 & BMP4 rs17563	1.474	1.27	1.711	**3.47E-07**	1.267	1.055	1.522	**0.0114**	2.438	1.798	3.307	**9.78E-09**
ROBO1 rs1387665 & BMP4 rs17563	0.76	0.653	0.885	**0.0004**	0.71	0.591	0.853	**0.0003**	0.853	0.627	1.16	0.3098
ROBO1 rs4513416 & BMP4 rs17563	1.268	1.089	1.477	**0.0023**	1.399	1.161	1.687	**0.0004**	1.096	0.813	1.478	0.5455

* Summary of significant cases-only interaction analysis between the BMP4 SNPs and SNPs from the RORA-ROBO1-HTRA1 triumvirate among all cases and wet (Neo) and dry cases specifically (significant interactions are in pink).

consistent results between the two statistical methods were unobtainable when significance was seen in one method. Regardless, the results tell us a number of things about the gene in relation to this disease. In looking just at the most significant SNPs in each of the cohorts, it appears that SNPs at and around LD Block 1 (Figure 5) increase the risk of AMD in all subtypes, while SNPs at and around LD Block 2 are protective against neovascular AMD or reduce the risk of all subtypes of AMD. Each cohort was associated with a different most significant SNP, and only rs17563 was at a significant level in multiple cohorts (NESC and Koreans). This reaffirms the concept that AMD may have different genetic risk factors in different populations. Interestingly, in addition to rs17563 which has already been discussed as a risk factor in other diseases, the most significant SNP in the GSSibs cohort, rs4898820, which decreased the risk of all AMD subtypes, has been implicated in the pathogenesis of adolescent idiopathic scoliosis (AIS) (Mórocz et a., 2011). This is an excellent example of the importance of understanding the multiple roles SNPs play in different diseases and in personalized medicine. If clinicians attempted to target this SNP as a treatment for AIS without understanding its protective benefits for AMD or the individual patient's overall risk of developing AMD, they might inadvertently increase the patient's susceptibility to one disease while trying to treat another. While many drugs and other treatments have side effects, it is important to balance the risks with the benefits, especially in regards to the disruption of vital functions, such as sight.

In the cases-only interaction analysis there were a number of interesting results. The BMP4 SNP rs17563 was associated with all four of the most significant interactions, two with SNPs in the RORA gene and two with SNPs in the ROBO1 gene. The two

most significant interactions were between BMP4 and RORA (rs730754, $p = 1.51e^{-5}$ and rs8034864, $p = 9.78e^{-9}$), and both interactions increased the risk of dry AMD. In contrast, both of the highly significant BMP4-ROBO1 interactions, rs4513416 and rs1387665, are associated with an increased risk of wet AMD ($p = 0.0004$ and $p = 0.0003$, respectively). These differences in risk factors between subtypes was seen throughout the analysis with six interactions only significant (or considerably more significant) in wet cases, and seven interactions only significant (or considerably more significant) in dry cases (Table 5). The only significant interaction that involved the HTRA1 gene (rs1049331) also involved the BMP4 SNP rs17563, and was only significant among cases of dry AMD. Variants within the BMP4 gene are clearly involved in all aspects of AMD pathogenesis and specific variants may be utilized as therapeutic targets for the specific subtypes of AMD.

These results may be highly informative in better understanding the genetic mechanisms behind the pathogenesis of AMD and the utility of BMP4 as a therapeutic target, and at first glance the results may also suggest the utility of the rs17563 SNP in the proposed OPLL-BMP4-AMD identification method, however, there is more to consider than just the surface results. The specific parameters of the hypothesis along with the details of the results and the various aspects of the analysis combine to form a much more complex picture. Issues such as study cohort, significance level, repeatability, and disease subtype influence how these results should be interpreted in relation to the practicality of the proposed identification methods. All these factors, along with the OPLL results presented in the next chapter will be discussed in relation to the proposed method in the OPLL-BMP4-AMD Identification Method section.

OSSIFICATION OF THE POSTERIOR LONGITUDINAL LIGAMENT

Introduction

The second important aspect of this research concerns ossification of the posterior longitudinal ligament (OPLL) in the cervical spine. The posterior longitudinal ligament runs along the back of the vertebral body, in the spinal column, from the second cervical vertebra (C2) in the neck to the sacrum of the pelvis, and is adherent to and sometimes blends with the hard outer covering of the intervertebral discs (Taguchi, 2006). Ossification, or the change of the ligament into bone, causes the ligament to expand beyond the normal anatomical bounds, where it firmly attaches to the vertebral bodies and discs (Epstein, 2002a). Ossification can impinge on the spinal cord causing a number of neurological problems (Singh et al., 2004). Diagnosis occurs via radiographs taken at clinics when patients suffering from neck pain come in seeking treatment (Figure 6).

OPLL has been a disease of significant interest in Japan for the last 40 years. As a result, until about five to six years ago, most of the literature on the disease was coming out of Asia, with a few sporadic reports from other countries. For a majority of this time period OPLL was considered to be a "Japanese Disease," as it was believed to reach its highest prevalence in this population (Matsunaga and Sakou, 2006a). Other Asian populations are thought to present with the next highest prevalence. However, OPLL prevalence in these populations is still quite low overall: below 10%, even in the

Figure 6. A classic radiographic example of OPLL. Image as seen in a clinical setting.

Japanese. Historically, OPLL is considered to be mostly anecdotal among European populations, with a suspected prevalence of 1.7% (Harsh et al., 1987; Koga et al., 1998; Saetia et al., 2011). More recently, investigators report that due to OPLL's association with other common bone diseases in Whites, the disease must have a higher incidence in the population than was previously thought, and similar demographic attributes as among Asians (Matsunaga and Sakou, 2006b; Kalb et al., 2011; Wang and Thambuswamy, 2011; Wu et al., 2011). Although, the overall prevalence is considered to be similar to that proposed by Koga and colleagues in 1998 (up to 1.7%).

The fact that OPLL is believed to be a rare disease among individuals with European ancestry, and most populations outside of Asia, makes it an ideal candidate for a forensic identification methodology. A rare skeletal manifestation such as OPLL has the potential of narrowing down possible matches in a candidate pool when trying to

identify remains. For the sake of this research, the proposed scarcity of OPLL among populations provides the tool for reducing candidates and improving matches, while the OPLL-BMP4-AMD connection provides the database from which to draw potential matches. This chapter will discuss the aspects of OPLL, the history of research concerning the disease, and present the results of the research conducted for this project to better understand the true prevalence of OPLL outside Asian populations.

Explanation of the Disease and History of Research

Interest in OPLL, especially ossification in the cervical spine, has a surprisingly long history. In 1838 CA Key reported that ossification of the spinal ligaments could be responsible for spinal cord paralysis and subsequent paraplegia (Matsunaga and Sakou, 2006a). The disease attracted further attention in 1960 when Tsukimoto presented a post-mortem case report where severe spinal cord symptoms were caused by ossification within the ligaments of the cervical spinal canal (Tsukimoto, 1960). Originally, the disease was termed "calcification of the posterior longitudinal ligament." However, after a pathology study a few years later showed that this condition involves true ossified tissue, "ossification of the posterior longitudinal ligament" was proposed as a name-change, and the disease has been known as OPLL ever since (Terayama et al., 1964).

Ossification of the cervical posterior longitudinal ligament represents a continuum of disease beginning with hypertrophy of the posterior longitudinal ligament (PLL) (Figure 7) followed by progressive coalescence of centers of chondrification and ossification (Bizri et al., 2009). The PLL is composed of two strata of fibers and runs from the C2 vertebra to the sacrum. The ligament is more firmly attached to the

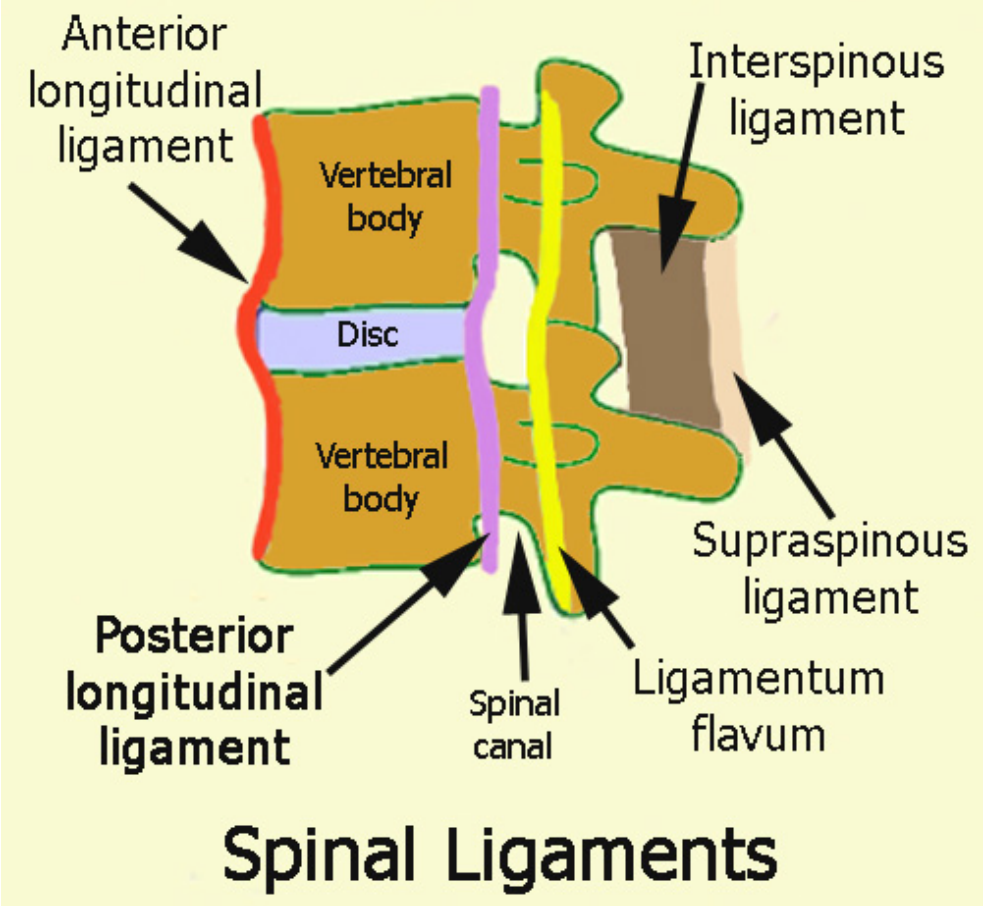

Figure 7. Location of the spinal ligaments. The PLL (purple) runs the length of the spinal column, and while this paper discusses the cervical spine, the spinal ligaments are show here in the thoracic region for clarity (From Wikimedia Commons).

intervertebral discs than to the vertebral bodies themselves, which is why in some cases OPLL seems to hover just over the vertebra (Figure 6). The PLL significantly differs from the anterior longitudinal ligament in the clinical sense as any alteration in the anatomy of the PLL can have severely negative effects on the spinal cord and nerve roots (Taguchi, 2006). The initial hypertrophy of the PLL which leads to ossification is attributed to fibroblastic hyperplasia and increased collagen deposition. This is followed by progressive mineralization and cartilaginous growth resulting in immature ossification centers, which later mature into Haversian canals, which are actively engaged in bone

marrow production (Epstein, 2002c). In addition to the work done by Terayama et al. (1964) into the pathology of the ossification, other research has confirmed the fact that OPLL is composed of osseous tissue derived from endochondral bone formation (Tsuzuki, 2006; Saetia et al., 2011).

Growth of the ossification averages 0.4 mm/yr in depth, and 0.67 mm/year in length (Epstein, 2002c). Although a high number of cases present asymptomatically, symptoms include sensory and motor dysfunction, abnormal reflexes, general problems with the nerves and spinal cord, and even neurological dysfunction (Epstein, 2002c; Chang et al., 2012). The most commonly reported symptom is myelopathy or pathology related to the spinal cord resulting from compression, which is of note as OPLL is often overlooked as an independent cause of myelopathic conditions (Hirabayashi et al., 1981; Harsh et al., 1987; Singh et al., 2004; Taguchi, 2006; Hsieh and Wang, 2011; Saetia et al., 2011). The average age of onset for the disease is around the 50 years, with the highest prevalence in the sixth decade of life (Nose et al., 1987; Kaneko, 2006; Kawaguchi et al., 2006; Matsunaga and Sakou, 2006b). Twice as many males present with the disease as females (Koga et al., 1998; Singh et al., 2004; Matsunaga and Sakou, 2006a; Wu et al., 2011; Jeon et al., 2012). About 70% of cases are localized to the cervical vertebrae, with the rest equally distributed throughout the thoracic and lumbar spines (Trojan et al., 1992; Epstein et al., 2002c; Stapleton et al., 2011). In effect, OPLL of the cervical spine is the primary research target for most investigators in the field. Four distinct types of OPLL were characterized and defined by Hirabayashi et al. (1981) and have become the standard for research. The four types (Figure 8) are continuous (27% of cases), segmental (39%), mixed (29%), and other (7%; Tanaka et al., 2006).

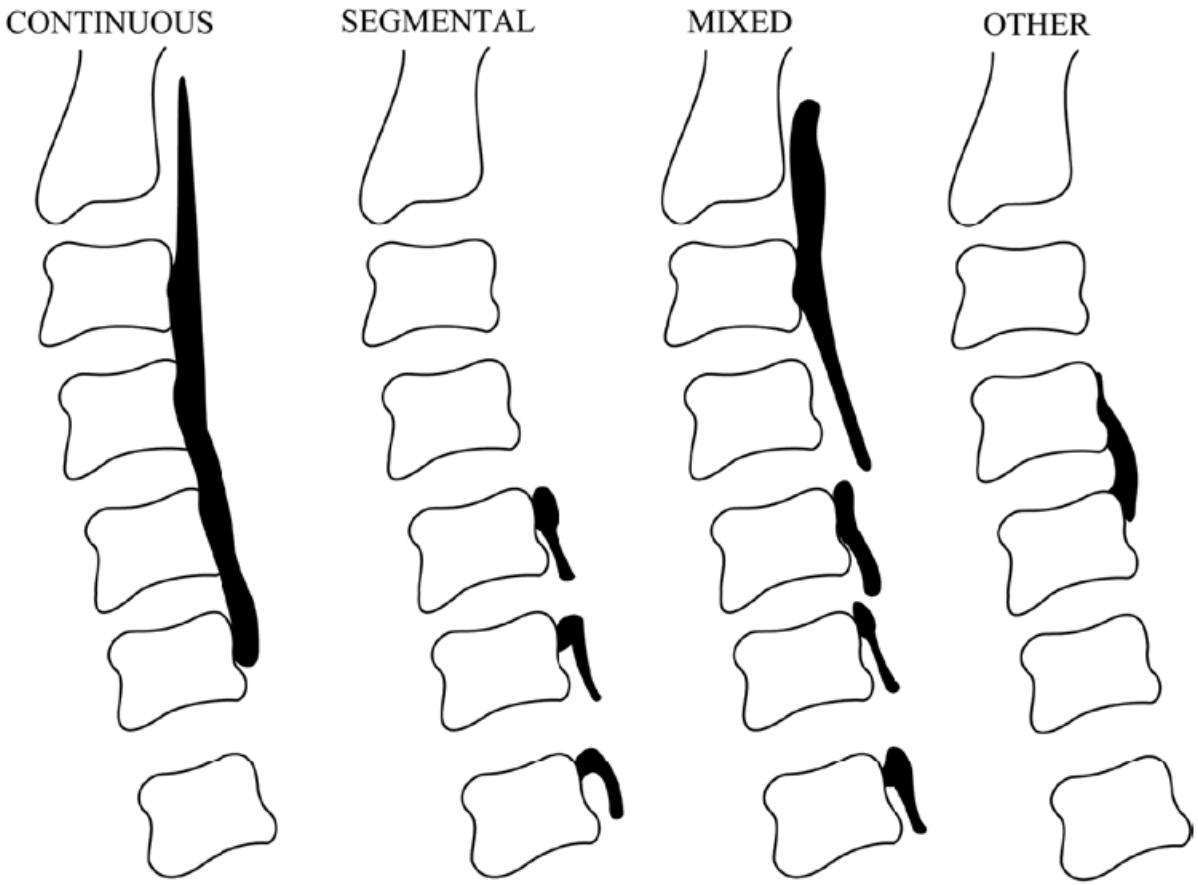

Figure 8. The four different types of OPLL. Drawn from a lateral view, vertebral bodies C2-C7.

The segmental type consists of one or more separate lesions behind the vertebral bodies, the continuous type is a long lesion extending over several bodies, the mixed type is a combination of segmental and continuous lesions, and the other type consists of lesions mainly posterior to the disc space (Hirabayashi et al., 1981; Nagata and Sato, 2006). The continuous type has been repeatedly seen as occurring most frequently at the levels C2-C4.

OPLL has been extensively studied in Asian populations with a proposed prevalence of up to 4.3% in Japanese, and more recently, a potential prevalence of up to 8.92% among Chinese (Ren, 2012a). Understanding the disease is of profound importance in Japan, and there are a number of committees dedicated solely to the improvement in practical and clinical knowledge of the disease (Nakamura, 2006). Consequently, the most informative literature is coming from this part of the world, although now there is a push to consider this disease as a possibility in every population. While there does appear to be a conscious move away from the notion that OPLL is a "Japanese disease," there is still little known about the specifics of the disease in non-Asian populations: although a peak prevalence of 1.7% has been reported in Whites (Koga et al., 1998). Harsh et al. (1987) reported that OPLL in his time was rare in North America, citing only 20 patients in the literature. A few years later, Trojan et al. (1992) claimed there were no incongruities between the presentation of the disease among Japanese and non-Japanese, based solely on eight case studies. But recently, Kalb et al. (2011) and a number of other researchers have tried to instill the idea that there is a higher prevalence of OPLL in European and North American populations than was ever previously considered (Wang and Thambuswamy, 2011). Today, there are a number of

institutions that treat OPLL in the United States, such as the Mayo Clinic, The Spine Institute in Los Angles, Irvine Orthopedic Associates of California, and a number of neurosurgery departments in institutions like the University of Utah. However, as OPLL is still not considered a prominent problem in the U.S., these clinics only treat on a case by case basis and do not report OPLL as a specialty. This fact suggests that while there is belief that OPLL prevalence in American populations is higher than the historically reported 1.7%, it is still rare enough to be useful for an identification method. Also, the interest in finding the true prevalence of OPLL in non-Asian populations makes the research undertaken for this project, and discussed below, pertinent not only to the forensic or anthropological communities, but to the medical community as well.

Etiology and Genetics

In spite of the long history of interest and research concerning this disease, the etiology of OPLL still remains unclear. Some suggested theories include an infectious etiology, fluoride intoxication, an immunological mechanism, diabetes mellitus, and even trauma, although none has gained acceptance (Singh et al., 2004; Jones et al., 2011; Chang et al., 2012). The concept of trauma-induced induction of OPLL has been a favorite theory with many researchers. However, studies have show that trauma or repeated mechanical stress, especially in the cervical spine, only serves to progress already existing OPLL or cause myelopathy in individuals already suffering from OPLL (Tanno et al., 2003; Jones et al., 2011; Chang et al., 2012). There is an understanding that the pathogenesis of OPLL is a complicated mixture of many genetic and environmental aspects, that as of yet, remain unclear (Karasurgi et al., 2013).

In contrast to the work done on the possible environmental factors of OPLL, research into the genetic factors which influence OPLL has been much more informative. There is a strong familial link to the disease, with 20-30% of relatives also having the disease, and 85% of monozygotic twins also having OPLL (Matsunaga and Sakou, 2006a; Stetler et al., 2011). In terms of genetic risk factors, a number of genes in the BMP family have been reported to have abnormal expression in relation to this disease (Stetler et al., 2011). Specifically, studies on BMP2, BMP4, and BMP9 have shown that these genes are localized near sites of ossification and contain SNPs which increase the risk of developing OPLL (Furushima et al., 2002; Hoshi, 2006; Wang et al., 2008; Meng et al., 2010; Ren et al., 2012a,b; Yan et al., 2013). The BMP4 gene, which was the inspiration for this research, has been long thought to have a significant part in the onset and extent of OPLL (Furushima et al., 2002; Tanaka et al., 2003; Yoshikawa, 2006). A study by Meng et al. (2010) concluded that male Chinese Han patients with the CT and TT genotypes at SNP rs17563 within BMP4 have a greater susceptibility to OPLL and have more extensive OPLL in the cervical vertebra of the neck. More recently, Ren et al. (2012a) looked at 18 SNPs within BMP4 and confirmed that rs17563 significantly increases susceptibility to OPLL in both males and females.

Genetic studies of OPLL have also revealed several gene loci outside the BMP family that may be involved in the pathogenesis of this disease. Genes encoding for proteins that process extracellular inorganic phosphate, collagen fibrils, and transcription factors involved in osteoblast and chondrocyte development and differentiation have all been implicated in the pathophysiology of OPLL (Stapleton et al., 2011). Genome-wide searches have identified chromosomes 1, 2, 6, 7, 11, 14, 16, 20, and 21 as possibly

housing genes which contribute to the susceptibility and progression of OPLL (Tanaka et al., 2003; Karasurgi et al., 2013). Specific genetic factors that have already been targeted for research and clarification include the Vitamin D receptor, Types VI and XI collagen receptors, the alpha B crystalline receptor, and the HLA complex (Koga et al., 1998; Bizri et al., 2009; Stapleton et al., 2011; Stetler et al., 2011). Research has also been conducted on the genes CDH13, COL6A1, COL11A2, IGF1, NPP1, OPN, PRG1, PTHR1, and TGFβ3, although none of these genes is thought to match the influence of BMP4 (Koga et al., 1998; Furushima et al., 2002; Tanaka et al., 2003; Stapleton et al., 2011).

Genetic research into the etiology of OPLL has also focused a great deal on the relationship between OPLL and two other ligament problems local to the spine. During the earliest research into OPLL, it was thought that this disease was a subtype of the condition known as diffuse idiopathic skeletal hyperostosis (DISH; Matsunaga and Sakou, 2006b). DISH is a syndrome which involves ossification of the soft tissue and ligaments near the ventral aspect of the cervical and/or thoracic spine (Mader, 2002). For anthropologists, the hallmark of DISH is the wax-like ossification that flows down the right anterior portion of the vertebral bodies. Today OPLL is considered to be its own disease, but it is reported to occur in about 50% of DISH cases, suggesting there must be a genetic link which induces ossification (Trojan et al., 1992; Koga et al., 1998; Saetia et al., 2011). The second disease of interest for OPLL researchers is ossification of the ligamentum flavum (OLF). OLF is a disease of ectopic bone formation within the ligamentum flavum (Figure 7), which may result in neurological compromise much like OPLL (Christiano et al., 2011). OLF is thought to mimic OPLL in that it has a high

prevalence among the Japanese and it has a male preponderance. OLF and OPLL are often discussed together because they both reside within the spinal canal, ossification of one is often associated with ossification of the other (suggesting a possible genetic link), and most importantly, any treatment must consider the effect on both ligaments (Iwasaki, 2006; Yoshida, 2006; Kotani et al., 2013). Thus, while OPLL is an independent disease, there are clearly strong genetic associations between the various intraligament ossification disorders of the spine.

Diagnosis and Treatment

Due to the nature of OPLL, all current clinical diagnoses are performed radiographically. Traditionally, plain radiographs and lateral or transverse x-rays were the preferred method of diagnosis (Koga et al., 1998; Yonenobu et al., 2006; Chang et al., 2012; Jeon et al., 2012). Today a number of other techniques are used in addition to the traditional methods, including CTs, MRIs, and diffusion tensor imaging which is believed to provide more information to clinicians when preparing surgical options (Tanaka et al., 2006; Jones et al., 2011). One aspect of diagnosis that has not changed over the years is the fact that radiographs are only taken when a patient comes in complaining of neck pain or presents with a number of the neurological and nerve symptoms associated with the disease. This fact is very important to the research described here as this method of diagnosis precludes the identification of earlier stage or minimal ossification that may not cause any problems, and excludes individuals who are asymptomatic and never have cause to seek treatment from the prevalence reports. Prevalence studies resulting from such diagnostic criteria have the drawback of representing only those actively suffering

from the disease, instead of all the individuals in the population who have OPLL, regardless of clinical presentation.

Treatment for OPLL is a sensitive and often challenging issue because of the ossification's location in an enclosed space and its proximity to the spinal cord. Since surgical treatment was first attempted in this area, there has been a great deal of discussion as to what form of surgery is best for what case, when in a person's life it should be performed, at what extent of ossification surgery is necessary, and how long the treatment can be expected to last (Epstein, 2001; Cardoso et al., 2011; Vedantam et al., 2011; Wu et al., 2012). Currently, there are two anterior and two posterior surgical approaches that have gained general acceptance in the clinical community (Epstein, 2002a,b). Unfortunately, neither approach has been overly successful and there are a number of complications that arise after surgery, including spinal cord injuries, general disability, and postoperative progression of the ossification (Ngata and Sato, 2006; Wu et al., 2011; Wu et al., 2012). Once again, there is an aspect of this disease which is discouraging in the clinical sense but promising in terms of the proposed identification method. Since surgical treatment of OPLL is often ineffective, it means that OPLL should be visible in the population at every stage of its development and throughout the foreseeable future, making it an identification target that could be used for some time.

Comparative Skeletal Analysis

Introduction

The second aspect of the research undertaken was the analysis of OPLL in an American skeletal population. Historically, interest in this disease has been centered in

Asia, especially in Japan, and little research as been done into the specifics of the disease among other populations, except for the few incidences discussed above. In addition, diagnosis of this disease is traditionally based on radiographic analysis of clinical patients complaining of spinal and/or neurological symptoms. As a result, diagnosis often occurs only in symptomatic individuals, and only after the disease has reached an extreme state. In order to better understand the prevalence of OPLL among American populations, how the presentation of the disease differs between radiographic and skeletal diagnosis, and the utility of this disease as an identification method in forensics, a sample of 1051 50+ year old White and Black males and females were analyzed from the Smithsonian's Terry Collection. The Terry Collection is currently comprised of approximately 1728 complete skeletons of individuals of known sex, age, ancestry, cause of death, and pathological conditions, spanning in age from 16-102 years who died during the 20th century (Hunt and Albanese, 2005). Individuals with missing or questionable demographic information were not used in this study.

Methods

The study population was composed of individuals 50 years and older because the average age of onset of the disease is around 50 years, and the highest prevalence of the disease is thought to occur in the sixth decade of life (Nose et al., 1987; Koga et al., 1998; Kaneko, 2006; Kawaguchi et al., 2006; Matsunaga and Sakou, 2006b). In addition, the cervical spine was specifically targeted for this study since 70% of OPLL cases occur in this location, and much of the specific research into this disease, including the genetic association of BMP4, has been linked directly to OPLL of the cervical spine (Trojan et

al., 1992; Tanaka et al., 2003; Nagata and Sato, 2006; Meng et al., 2010). Thus, the study population consisted of all cases in the Terry collection which were identified as 50 or older and had a complete cervical spine (C-spine). A list of all pertinent case numbers was compiled and to avoid bias in the initial analysis, defining features in the case record, such as sex, age, and ancestry, were blocked out until after all the cases were examined. A total of 1060 cases were available for analysis according to the records. An initial examination of the collection identified six cases where the C-spine was missing or incomplete and three cases where the individual was actually of Asian ancestry, leaving 1051 Black and White individuals with well preserved C-spines for analysis.

For each case, the entire cervical spine was examined for the presence of OPLL. If an entire C-spine was free of ossification (Figure 9) than it was marked as "absent" and

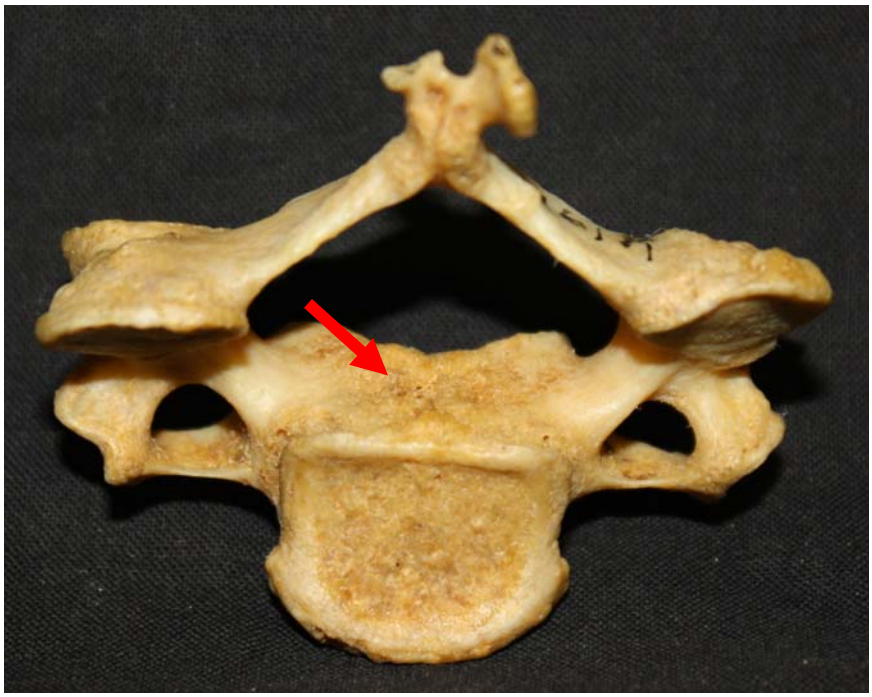

Figure 9. Terry Collection vertebra with no ossification. The red arrow points to the flat surface of the vertebral body. View is posterior-inferior.

set aside. Only if ossification was present, even on just one vertebra, was further information collected and additional analysis conducted. OPLL was considered present if the telltale ossification on the back of the vertebra was visible or palpable, at which point information was taken on the type, location, and extent of the ossification (see Appendix B). Identification through palpation was shown to be sensitive down to a 10^{th} of a millimeter in thickness. Measurements were taken to the nearest 100^{th} of a millimeter with a digital caliper at the maximum length, width, and depth of ossification (Figure 10). Photographs were taken throughout the analysis to exemplify the variability of the presentation of OPLL throughout the collection and between the various subgroups of interest (Figure 11).

Figure 10. Analysis table and measurement technique.

Figure 11. Case 152. An example of ossification on every vertebra in the C-spine. View is posterior-superior.

After all the prevalence data, measurements, and photographs were completed, the sex, age, and ancestry for each case was revealed and cases were grouped by sex, age, ancestry, and sex/ancestry for analysis. Both qualitative and quantitative assessments were carried out on the data. Statistical tests were conducted using the statistical package R version 2.15.1 (2012-06-22) to identify differences in prevalence and extent of ossification by group. Results were then compared to previously accepted aspects of the disease to note any differences or deviations from currently accepted demographics of OPLL among populations.

Results

Prevalence. The overall prevalence in the study population was much higher than anything previously reported on either Asian or non-Asian populations. Of the 1051 individuals studied, 542 presented with OPLL for an overall collection prevalence of 52%. In the various subpopulation groups in the study, there were likewise interesting, and highly unexpected results ranging from 32-67% prevalence (Figure 12). Ossification occurred in 299 of 617 cases (48%) in Whites, 244 of 435 cases (56%) in Blacks, 372 of 587 cases (63%) in males, 171 of 464 (37%) in females, 213 of 351 cases (61%) in White males, 86 of 266 cases (32%) in White females, 159 of 237 cases (67%) in Black males, and 85 of 198 cases (43%) in Black females. Among the specific age groups the prevalence was as follows: 55% in the 50s (N=324), 54% in the 60s (N=357), 48% in the 70s (N=252), 47% in the 80s (N=105), 20% in the 90s (N=15), and 100% in the over 100 group. It should be mentioned that the 100+ group only consisted of two individuals, both Black females, and all data from this group should be considered anecdotal.

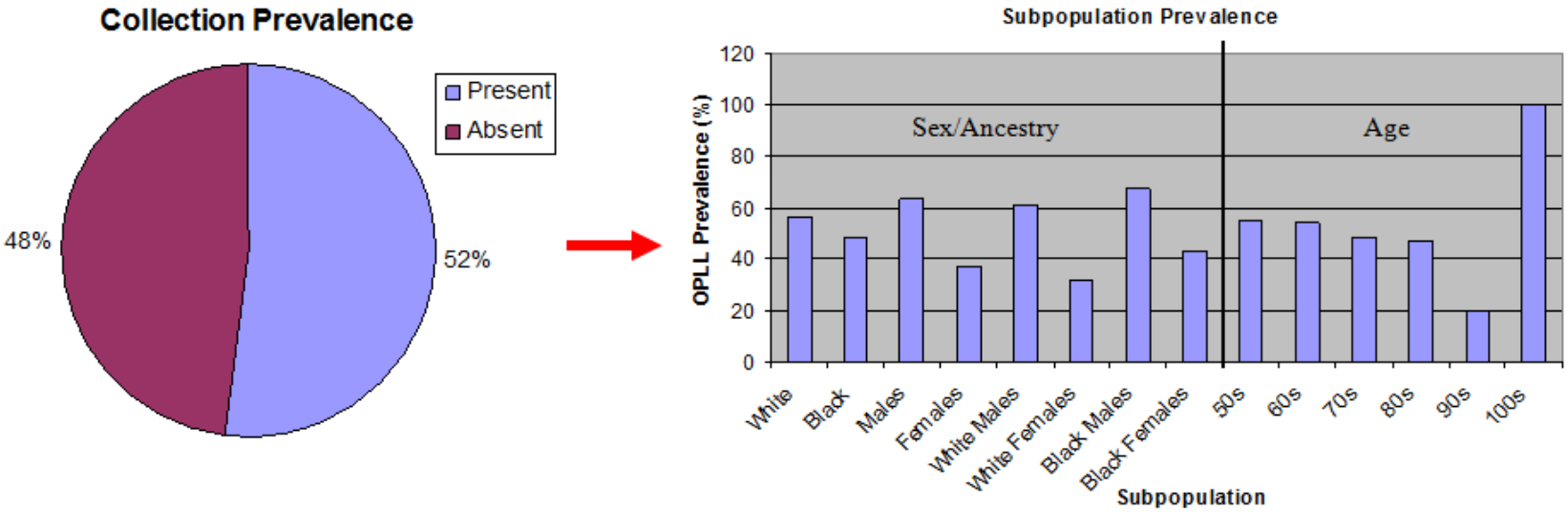

Figure 12. OPLL prevalence in collection by group. Prevalence shown in the entire collection and the various subpopulations.

Groups were compared for differences using Pearson's Chi-Squared tests at the $p = 0.05$ level. The prevalence among males is significantly higher than among females ($p < 2.2x10^{-12}$), significantly higher among White males compared to White females ($p = 2.98x10^{-12}$), and significantly higher among Black males compared to Black females ($p = 4.28x10^{-7}$). There is a significant difference between White and Black females ($p = 0.0193$) and between all Whites and all Blacks ($p = 0.0147$), but there was no significant difference between White and Black males. When comparing all age groups there was a small difference between the groups ($p = 0.028$), but when the 90s and 100s were removed from the analysis for small sample size (only a total n=17), there was no apparent significant difference ($p = 0.2021$).

Type. Three of the four types of OPLL were witnessed in the study population: segmental, continuous, and mixed (Figure 13); type "other" was not seen. Almost all of the cases were segmental (98.90%) with two examples of continuous OPLL (0.37%) and four cases of mixed OPLL (0.73%). The two continuous cases were White individuals, one male and one female. The mixed cases included two Black males, one White male, and one Black female. Statistics were not performed between groups with this aspect of the data due to the very small number of cases which were not of the segmental type. The results would have appeared significantly different because of the small sample, regardless of any actual significance.

Location. There were 32 different location combinations (Figure 14) in the population with the most common location being cervical vertebrae C3-4 (125 cases). The most common vertebrae involved were C3, C4, and C5, respectively. Segmental cases occurred in all parts of the C-spine while the continuous type was found primarily

Figure 13. Examples of OPLL types seen in the Terry Collection. A: Segmental, B: Continuous, C: Mixed.

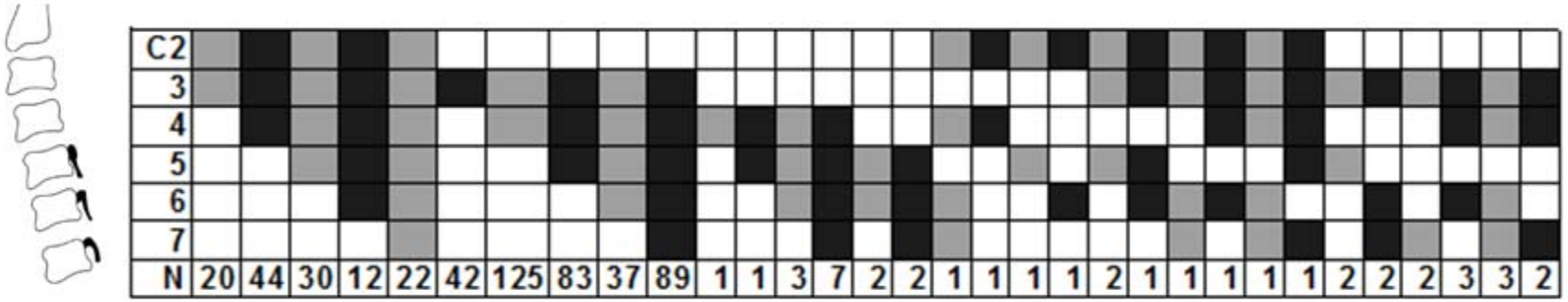

Figure 14. Location of ossification in the Terry Collection. Combinations seen on cervical vertebrae C2-C7. Black and grey coloring is only used to differentiate between adjacent location combinations.

in the upper half of the C-spine.

Extent. Most cases of OPLL involved ossification over 2-5 vertebral bodies, with the average being 3.2 vertebrae. The area of ossification on the most ossified vertebra ranged from 3.53 to 255.35 cubic millimeters. The average area of ossification on the most affected vertebral body for the major subpopulation groups ranged from 51 to 65 mm^3: 62.31 mm^3 in Whites, 62.55 mm^3 in Blacks, 65.69 mm^3 in males, 55.33 mm^3 in females, 66.75 mm^3 in White males, 51.29 mm^3 in White females, 64.24 mm^3 in Black males, and 59.40 mm^3 in Black females. Mean area of the groups were compared by using a combination of the F-test to compare two variances and a two sample t-test to test for a true difference in means. There was no significant difference in the mean area between Whites and Blacks, between White and Black females, White and Black males, or Black males and females. There was a significant difference in area between all males and all females ($p = 0.0125$) and between White males and White females ($p = 0.0067$), suggesting that the majority of the male-female difference is coming from the discrepancy in the White population.

Among the age groups, the area of ossification was highest among those in their 90s with an average of 84.97 mm^3, followed by those individuals in their 70s, with an average ossification of 72.99 mm^3. When taken as an entire collection, there were no significant differences in mean ossification associated with age group.

There is a wide range of ossification extent throughout the collection (Figure 15). Some cases presented with wide and relatively flat ossification, other cases presented as thin and deep, and there were cases that presented with every combination in between. There was no pattern which was significantly more common to any one subpopulation.

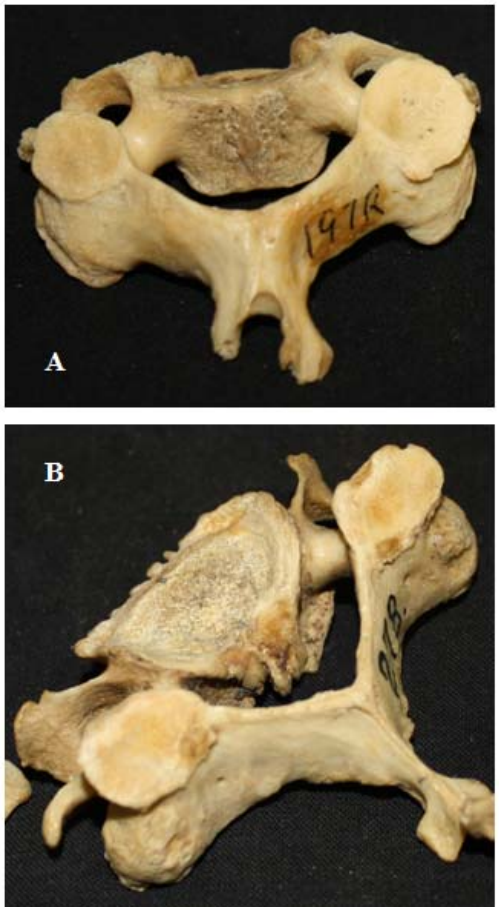

Figure 15. Variation of ossification seen in the collection. Image A is an example of a White female with an overall small area of ossification that is primarily flat. Image B is an example of a Black male with a large area of ossification that is both wide and deep.

However, as can be expected by the statistical differences of ossified area between male and females, the females qualitatively seemed to present more frequently with the thinner and flatter ossification patterns.

Discussion

The results from this research conducted on OPLL in White and Black populations strongly contradict what was previously understood about the prevalence of this disease in non-Asian populations. The prevalence range witnessed (32 – 67%) brings

into question not only the past assumptions about American population OPLL prevalence, but also the prevalence of the disease in Asian groups as well. One of the key differences is that this research was conducted on a skeletal collection, whereas OPLL is traditionally diagnosed through radiographs of patients seeking treatment for neck or neurological complaints. Skeletal assessment of the disease allows for an understanding of the true number of potential sufferers in the population, whether or not they are currently symptomatic. Ossification was recorded at a sensitivity of 0.10 mm, a depth that would be difficult to see on radiographs and most certainly not cause symptoms. Thus, this study identified a wider range of OPLL cases than is currently seen by clinicians.

As a result of using a skeletal population for analysis and such a sensitive degree of identification of ossification, there is the concern that the high prevalence witnessed here is an artifact of an overabundance of early stage or minimal ossification. Taking into account Epstein's 1994 paper suggesting an early form of OPLL, which she termed "OPLL in evolution" (OEV), and the measurements of the spinal canal, a reassessment of the data was conducted to quell such concerns. Research into the spinal column has shown that the average diameter of the normal spinal canal in the cervical spine is 13-15 mm, and the diameter of the spinal cord can very greatly in this region, from 8.8 to 14 mm (Sherman et al., 1990; Morishita et al., 2009). Consequently, it was proposed for this follow-up analysis that any ossification less than 1 mm in depth would be considered an early stage, or OEV, and be taken out of the dataset. The depth of 1 mm equates to at least 2.5 years of ossification growth and, according to the diameter of both the spinal cord and spinal canal, has the potential to impinge on the spinal cord causing adverse

reactions and classic OPLL symptoms (Epstein, 2002c).

In the modified dataset, there were a total of 769 individuals, 456 White and 313 Black. The same analyses were carried out in terms of prevalence to show that, even when removing OEV cases which would most likely never be seen by a clinician, the prevalence witnessed in these populations is much greater than has been reported or suspected anywhere in the world. Overall, the modified collection population had an OPLL prevalence of 34%, down from the 52% seen when the OEV cases are added, but still much higher than the anticipated 1.7%. Ossification occurred in 138 of 456 cases (30%) in Whites, 122 of 313 cases (39%) in Blacks, 187 of 403 cases (46%) in males, 73 of 366 (20%) in females, 106 of 244 cases (43%) in White males, 32 of 212 cases (15%) in White females, 81 of 159 cases (51%) in Black males, and 41 of 154 cases (27%) in Black females. Among the specific age groups the prevalence was as follows: 33% in the 50s, 37% in the 60s, 33% in the 70s, 31% in the 80s, 14% in the 90s, and 100% in the over 100 group. Again, the 100+ group only consists of 2 Black females with significant ossification, and should only be discussed anecdotally. A comparison of the original cohort compared to the modified cohort and previously published results can be seen below (Figure 16).

Groups were once again compared for differences using Pearson's Chi-Squared tests at the $p = 0.05$ level. The prevalence among males is still significantly higher than among females ($p < 3.78 \times 10^{-8}$), significantly higher among White males compared to White females ($p = 1.087 \times 10^{-6}$), and significantly higher among Black males compared to Black females ($p = 0.0033$). There is a significant difference between White and Black females ($p = 0.02697$), but there was no significant difference between White and Black

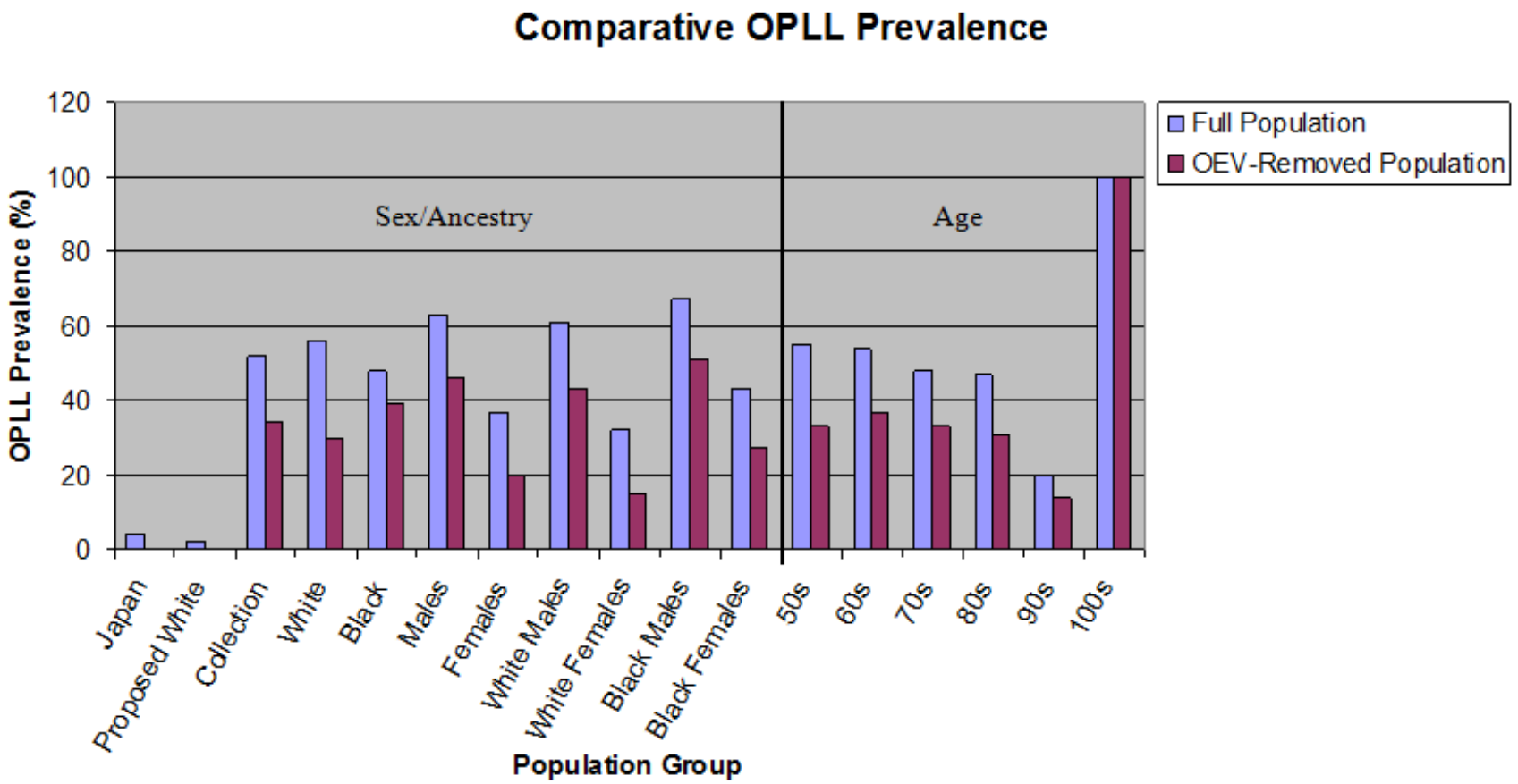

Figure 16. OPLL prevalence in the collection with and without OEV cases. Comparison of the two study populations and the currently accepted prevalence for the Japanese (proposed world high) and the proposed White prevalence. (N=2 in 100s group).

males ($p = 0.3743$), and no significant difference between all Whites and all Blacks ($p = 0.07919$). Among the age groups, there was no significant difference between all the groups ($p = 0.6344$), and when the 90s and 100s were removed from the analysis for small sample size (only a total n=17), there was still no apparent difference ($p = 0.8631$). There was only one significant change in the data as a result of the OEV modified analysis; the difference between the Black and White populations was lost. Otherwise, the only changes were prevalence percentages and a decrease in the significant p-values among groups. Regardless, this confirms the importance of these results and this data in understanding the true prevalence of OPLL in Black and White populations.

There were a number of other features of the disease that this research was also able to explore. Previously stated observations of a male bias were confirmed with OPLL appearing twice as often in all males in this sample than in all females. This male predominance was also witnessed independently within both the Black and White populations, although it was more pronounced between the White males and White females (61% vs. 32% in the full collection, and 43% vs. 15% in the OEV-removed population). In addition, research confirmed the previous reports that ossification is most common over 2-5 vertebrae, with an average of 3 vertebrae. Historically, OPLL is thought to present most commonly on C4, C5, and C6 respectively, and was witnessed here mostly on C3, C4, and C5. This data is similar enough to suggest that perhaps the vertebrae most involved in ossification vary by ancestral population or patient population.

Many more segmental cases were seen than is suggested by the literature, but this may be another unexpected result of looking at a skeletal collection. It has been reported that up to 50% of cases may have separation between the ossified ligament and the

vertebral margin, which gives the typical appearance of a sharp and thin radiolucent line between the OPLL and the vertebra in radiographs (Enzman et al., 1994). Therefore any ossification that was separated from the vertebral body by connective tissue, venous plexus, or crossed the intervertebral space, may have been forcibly and unintentionally removed during processing. With awareness that OPLL has a significant prevalence in non-Asian populations, new or more careful maceration and skeletonization techniques can be implemented to preserve this ossification.

In the age group analysis, there appears to be a negative correlation between age and incidence in the full population and somewhat of a bell curve in the OEV-removed population (Figure 16). This phenomenon will require more study in the future, but it is most likely a combination of a number of factors. Such as the issue that males have twice the prevalence of females and are historically believed to die at a younger age, for example, 71% of the 80s group is comprised of females. Thus, as the population ages, the individuals providing the majority of the prevalence in the population are dying younger and the overall prevalence can not help but decrease. In addition, it may be that individuals with OPLL also have other diseases which increase mortality with age, however, the mortality rates of individuals with OPLL is currently unknown. At the moment, this observation is more of an interesting aside until more research can be undertaken to better understand the underlying mechanisms influencing age group prevalence.

Lastly, these results do not seem as unexpected in comparison to previously reported prevalence when one considers the autopsy data coming out of Japan. In looking at 350 cases, Tsuzuki (2006) reported a 20% incidence of OPLL during autopsies

in patients 60 and older, regardless of any clinical findings of the disease during life. If more autopsy reports and skeletal collections are explored for OPLL prevalence, it is likely that the true prevalence of OPLL among all world populations would be better understood, and would be congruous with the data presented here.

Much of this data and analysis has provided indispensible information for the utility of the proposed identification technique in this research. How these results affect the technique and what future analysis may be needed is discussed in the next section. However, it is clear that this research produced a number of highly unexpected results that will have profound impact far afield from the original intent, and regardless of OPLL's value as an identification method. The understanding that a disease is rare in a certain population may preclude a doctor from diagnosing it, but this research suggests that OPLL is anything but rare outside of Asian populations and should be re-evaluated in both the anthropological and medical communities.

THE OPLL-BMP4-AMD IDENTIFICATION METHOD

Introduction

The research carried out for this project has greatly contributed to the general understanding and presentation of AMD and OPLL. However, the primary intent of this research was to consider the utility of the OPLL-BMP4-AMD relationship as a forensic identification method. On any given day in the United States, there are over 100,000 active missing person cases and 40,000 unidentified sets of human remains. The National Institute of Justice has deemed this "The Nation's Silent Mass Disaster" based on the fact that in a single year there are more individuals left unidentified than there were in the aftermath of the 9/11 attacks (Ritter, 2007). Moreover, there is not nearly the same level of effort being put into the investigation of these 40,000 individuals as there was for the World Trade Center disaster or Hurricane Katrina. Providing new methodologies for identification, such as the research presented here, can help in identifying these unknown individuals, and bring closure to their families. The basic elements of a skeletal profile, DNA, and fingerprint identification are useful to a point. However, when these methods are implemented and identification is still not possible, new analyses must be considered.

Anthropologists and other members of the forensic community must constantly strive to develop new methods and techniques that can help in the identification process. Biological profiles, compiled by anthropologists, are most helpful to investigators when there is a pool of possible candidates to compare these profiles, such as the missing

persons or Department of Motor Vehicles (DMV) databases. But without outside leads, it is often difficult to find comprehensive and appropriate databases for comparison so that the resulting potential matches are strong enough, or are comprised of a reasonable number with which to advance the investigation. Medical patient databases, especially those relating to specific diseases such as AMD, are a resource that provide considerable data on patients in addition to their disease state and allow for a more narrow comparison pool for the biological profile.

The operating hypothesis for this research is that given the genetic association between AMD and OPLL through the BMP4 SNP rs17563, the identification of OPLL on a set of remains would be the first step of a new method of identification through the genetic link of disease phenotypes in American Whites, and to a lesser degree, American Blacks (Figure 17). Identification of OPLL would initiate a genetic search for the risk variants among all AMD subtypes to confirm the individual was at a high risk for AMD. With that confirmation, local AMD databases could be searched with the use of the biological profile to create a subset of potential matches that could aid law enforcement officials in there pursuit of identification.

This section will discuss how the results of the various research aspects of this project inform the utility of the proposed identification method, and the practicality of using genetically linked diseases as a way to increase the likelihood of identification through improved potential match pools. While the exact parameters of the project hypothesis were not confirmed through the research, there are a number of positive results which will direct future OPLL-AMD research and have brought to light important assumptions and issues inherent in any multidisease based identification method.

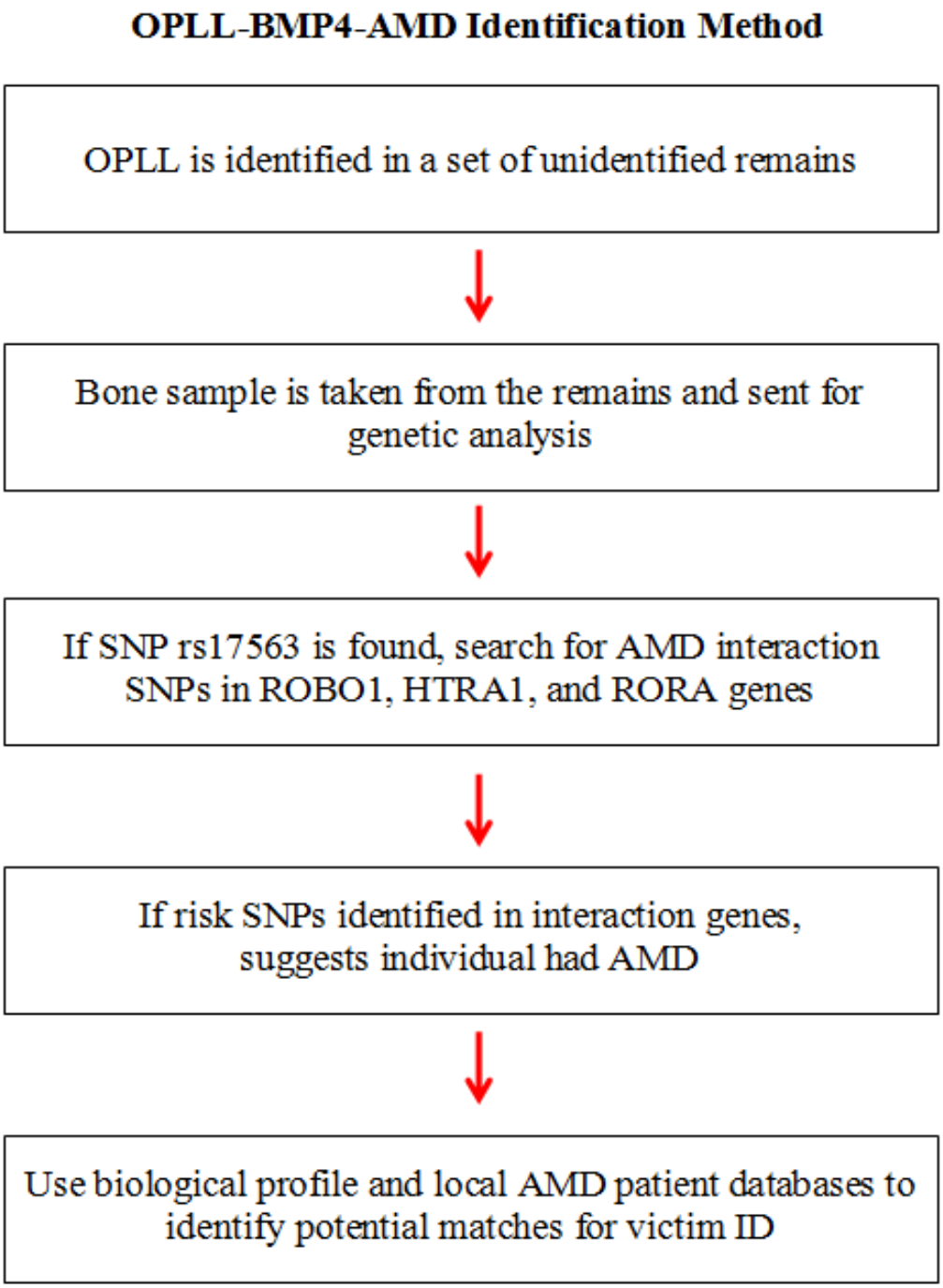

Figure 17. The OPLL-BMP4-AMD Identification Method flow chart.

Applicability of the Method

The applicability of this identification method, in terms of the research presented here, is defined by the parameters of the proposed hypothesis and the requirements of effective identification techniques. The hypothesis stipulates that 1) the SNP rs17563 is the risk factor for and genetic connection between OPLL and AMD, 2) rs17563 increases the risk of all AMD subtypes, 3) rs17563 interacts with other risk factors for AMD, especially in the RORA-ROBO1-HTRA1 triumvirate, and 4) all the rs17563 associations are significant in at least the American White population. Finally, for this to be a successful method, the skeletal disease, in this case OPLL, must have a small enough prevalence to be helpful in narrowing down the potential population pool.

Starting with the exploration of rs17563 as a risk factor for AMD, it was found that this SNP was in LD with another SNP, rs12898159, in the NESC cohort. However, the r-value was too low to assume complete linkage between these two variants. In effect, for the sake of this research, only rs17563's influence on the pathogenesis of AMD is of interest. The SNP rs17563 was found to increase the risk of all AMD subtypes ($p = 0.0161$) and dry AMD specifically ($p = 0.0098$), in the NESC cohort and the Korean cohort, respectively. Since the NESC cohort is comprised of all White patients it may appear as though the results have confirmed rs17563 as an overall AMD risk factor in the American White population. However, the fact that rs17563 was only found significant in one out of the two statistical testing methods, and that it was not significant in either of the other White groups suggests that the findings lack the repeatability and reliability necessary for an identification methodology.

In regards to the cases-only interaction analysis, the results were much more complicated in terms of analysis. The SNP rs17653 was associated with the four highest interaction analyses, two with RORA in dry patients ($p = 9.78x10^{-9}$ and $p = 1.51x10^{-5}$), and two with ROBO1 in wet patients ($p = 0.0003$ and $p = 0.0004$). However, in order to get large enough n-values for the subtype analyses, the cases from all the cohorts were pooled together as one population. This technique has some drawbacks, as the White cohorts were intermixed with the Korean cohort, the Korean cohort was the largest cohort in the study (almost doubling the largest White cohort), and in terms of the highest interaction analyses, only 25-31% of the cases in any population were dry AMD (in fact, the White GSSibs cohort had no dry cases). This brings into question which groups are actually influencing the interaction between rs17563 and the other risk genes, as only the NESC and Korean cohorts showed this SNP as an independent significant risk factor for AMD. On the other hand, it is a positive result that rs17563 was the only SNP to interact with all three risk genes tested (RORA, ROBO1, and HTRA1), and also had significant interactions in the wider AMD population as well as in each subtype analysis. There is still the question of whether or not the interaction analyses results would remain significant if the Koreans were removed from the analysis.

Lastly, there is the consideration of the prevalence of OPLL in the White population. This research has shown that the long held assumption that OPLL only reaches a prevalence of 1.7% among White populations is clearly flawed. OPLL is quite prevalent among White skeletons analyzed, with females presenting at 32% and males at 61%. Unexpectedly, it was even more prevalent among the Black population, with results reaching 43% in females and 67% in males. The combined prevalence in the

White population of 48% no longer makes OPLL an attractive target for an identification method for an entire ancestral population. Even when considering the OEV-modified population, the prevalence among Whites was still 30%. The presence of OPLL itself could discount about half to two-thirds the population, and the addition of the biological profile can pare down the potential matches even further, but it would not be as effective a tool as the project hypothesis suggested or this researcher had hoped for.

In taking into account the aspects of the hypothesis as it was originally presented, and the need for small prevalence in disease-based identification methods, this research was unable to support the hypothesis with the current dataset. Thus, the proposed OPLL-BMP4-AMD forensic identification method cannot be considered a viable technique in its current form. One of the biggest concerns, which is a factor in both genetics and forensics, is repeatability, and unfortunately the genetic data did not have the consistency that would instill confidence in the current application of the method. However, the results of this project do suggest that with an alteration of the hypothesis, and some additional research, there may be hope that in the future a version of this method will be helpful in solving the unidentified persons problem in the United States.

Future Research

The main concerns of the current study are the variable results of the genetic analysis and lack of effectiveness of OPLL in significantly reducing the population pool. Both of these matters can be addressed as research into this method moves forward. A reformatting of the hypothesis is imperative before any future work can be considered, but there are many ways in which the current hypothesis can be improved. For instance,

a different population of interest can be selected besides all American Whites who suffer from any form of AMD. Considering the results presented here, potentially focusing on only individuals who suffer from dry AMD may provide improved results, as the highest interaction analysis was in association with this subtype of patient. In addition, White females, especially White females in the OEV-modified group (prevalence of 15%), could be targeted specifically as they have the lowest prevalence of OPLL in all the groups studied. Perhaps this method works best on just a small subgroup of individuals as opposed to an entire ancestral population or sex. Since the Koreans also showed significance of rs17563 as a risk factor for AMD, it may be tempting to consider an entirely new ancestral population for research, such as an Asian group. However, recent research into the prevalence of OPLL in a Thai population suggests that not only is OPLL prevalence higher in Asian populations, it is more severe as well (Figure 18).

Figure 18. OPLL in a Thai population. A C3 vertebra from a Thai male with extensive and severe OPLL.

Besides changing the parameters of the hypothesis, additional research into the genetic aspect of the method would be beneficial to the project. Taking another look at the interaction analysis data and removing the Korean cohort may provide a clearer picture as to the interaction between BMP4 and the members of the RORA-ROBO1-HTRA1 triumvirate. This in turn may help inform whether looking specifically at a subtype of the disease, such as dry AMD, would be beneficial for the method. Also, it would be interesting to look at the independent risk of AMD and the interaction of the rs17563 SNP in a Black population. Even though AMD is much less common among African Americans, a comparison of the risk factors involved may still be informative in a comparative capacity to see if genes are being expressed differently across populations.

Finally, once all the adjustments are made, the new analysis is completed, and hopefully an adjusted method seems viable, it would be beneficial to apply the method, from start to finish, on a study population. Looking at both OPLL and AMD in an unknown population will help clarify the practical genetic connection between the two diseases, the ease of the method, and effectiveness of the resulting population pool. Even though the hypothesis discussed in this research was unsuccessful, the potential for future research suggested by these results support the general utility of a multidisease based identification method, and likelihood that a form of the OPLL-BMP4-AMD identification method may one day be feasible.

Assumptions and Issues Associated with the Method

Although there is promise for this work, there are a number of assumptions and issues associated with this method of identification that must be discussed and addressed

before moving forward with this line of research and other similar disease association studies. To begin, the method requires concurrent phenotypic expression of the diseases. If expression of the diseases occur at different points in the life cycle, then the method would be ineffective in confirming the presence of one disease with the identification of the other. This fact was one of the advantages of working with OPLL and AMD as both diseases appear during midlife and reach their highest prevalence after the age of 50. Thus, for the sake of the method, identification of OPLL in a set of remains already suggests that the individual was older and in the right age group to present with AMD.

It was assumed for this study that the deceased individual was aware of and had sought treatment for the medical disease, in this case, AMD. While this may be a problem to consider in future association studies, it did not seem like a stretch in the case of AMD. Since eyesight is such a vital part of one's everyday life, it is not overly ambitious to assume that *any significant* loss or alteration in one's central vision would prompt a visit to the ophthalmologist, even for the most obstinate individuals. The one issue that may impede treatment is economic status. However, this study population was by definition, composed of individuals who had sought treatment for the disease. Economic status as a hindrance to medical treatment and inclusion in the AMD databases is a factor which should be explored further as new research into this method is undertaken.

Another issue is disease prevalence. If a disease is very common in the local population, then using a disease-specific database as a comparison sample may not be any more informative than the local DMV records, for example, and potentially more troublesome. This was the exact issue encountered as a result of the OPLL prevalence

study conducted on the skeletal population. On the other hand, a very rare disease, while tempting to consider as a useful identification factor, can prove problematic if there is not enough background research to facilitate an association and only a few institutions throughout the country which treat the disease.

The issue of locally available treatment centers for the disease of interest presents yet another potential problem. The use of medical databases local to the recovery scene would be ideal, based on the assumption that the decedent was a local inhabitant and sought treatment for the disease at a treatment center reasonably close to his or her home. This is a credible assumption in the case of AMD due to its high prevalence, and it reduces the comparison pool to one or a few local medical institutions.

One of the biggest concerns encountered with this method is the issue of confidentiality on both the part of the individual patient and the larger institution. Maintenance of privacy and confidentiality when it comes to patient health information is a must. Fortunately, the Department of Health and Human Service's Health Insurance Portability and Accountability Act (HIPAA) already stipulates regulations for the disclosure of personal health information to law enforcement officials (Figure 19). It states that the disclosure of distinguishing physical characteristics, age, name, and contact information are permitted for the purpose of identifying or locating individuals such as missing persons. This in itself would suggest the cooperation of medical institutions and the protection of patient privacy. Moreover, informal discussions conducted with patients over the course of this study, suggest that they would be willing to allow disclosure for identification purposes, even in lieu of the HIPAA rule, as long as there is a stipulation in the paperwork at the onset of treatment.

To respond to a request for PHI for purposes of identifying or locating a suspect, fugitive, material witness or missing person; but the covered entity must limit disclosures of PHI to name and address, date and place of birth, social security number, ABO blood type and rh factor, type of injury, date and time of treatment, date and time of death, and a description of distinguishing physical characteristics. Other information related to the individual's DNA, dental records, body fluid or tissue typing, samples, or analysis cannot be disclosed under this provision, but may be disclosed in response to a court order, warrant, or written administrative request (45 CFR 164.512(f)(2)).

Figure 19. An excerpt from the HIPAA Privacy Rule. This passage details the disclosure of personal health information (PHI) to law enforcement officials.

Lastly, there will be cases in which the individual does not present with the bone disease, and thus this technique becomes irrelevant. However, there is no method employed by physical anthropologists that can be used in every case or on every set of remains. But, when this method is an option it has the potential of significantly cutting down the pool of potential matches and increasing the probability of identification. The overall results on the applicability of the OPLL-BMP4-AMD manifestation of this method may not have been overly encouraging, but with some alterations to the research parameters and additional analysis, it may yet prove as a valuable resource for forensic anthropologists.

CONCLUSIONS

Interdisciplinary Research

Interdisciplinary research is a widely utilized form of scientific inquiry. Collaboration provides new perspectives on long standing hypotheses and allows for results which may have substantial impact on topics far beyond the researcher's primary field. Unfortunately in forensic science, interdisciplinary research is often diminished to interspecialty research. While anthropologists consult with engineers and chemists, they usually do so under the umbrella of "forensically trained specialists." Moreover, the only medical personnel a forensic anthropologist may encounter on a regular basis is the medical examiner (ME), coroner, or pathologist in their jurisdiction.

Anthropologists bring much to the medico-legal process when dealing with skeletonized remains, because unlike MEs, they have the ability to procure identifying information from highly desiccated remains, such as age, sex, ancestry, and any unique bone pathologies. The techniques employed by anthropologists to assess this information would benefit greatly from collaborations outside of the wider forensic community. Work undertaken with medical professionals, or even research informed by other disciplines, has the potential of providing new avenues of forensic identification and results which may in turn significantly influence the fields from which anthropologists have consulted.

The work presented here is an exemplary step in the direction of forensic-medical collaboration for the improvement of identification methodologies. This research was initiated through genetic analysis in a medical research lab and significantly informed by the clinical literature, with the intent of establishing an anthropological identification method. The multiple perspectives helped to address the potential challenges associated with the method. Moreover, due to the many disciplines involved in the undertaking of the project, the results provided considerable advancements in understanding the diseases involved in this project, far beyond the scope of the method itself.

The discussion of this project is not complete without addressing the contributions of this research to each of the aspects involved, as well as the utility of the proposed method itself. This research grew beyond the bounds of the original hypothesis to influence all the areas which informed its creation. The OPLL-BMP4-AMD identification method, as it was originally proposed, may not be a currently viable method for identification, but the knowledge acquired in the analysis suggests that future research could provide a functional method, and that there is much more to the diseases explored than was understood at the project's inception.

BMP4 and AMD

BMP4 is a gene associated with many diseases in a number of diverse populations, and has been identified in the RORA-ROBO1-HTRA1 network as conferring a risk for AMD. Interaction analysis of BMP4 and the AMD-risk triumvirate confirms the fact that BMP4 is being regulated in this pathway. Moreover, its importance in the pathway is a factor of its functional, rather than genomic attributes. There is much

more to the BMP4-AMD association than has ever been considered before. The strong association between BMP4 and the dry form of AMD, as well as the differential interaction between BMP4 and the ROBO1 and RORA genes, suggests that BMP4 should start to be considered on par with the RORA-ROBO1-HTRA1 triumvirate in future research into the pathogenesis of AMD.

Variants in the gene can both increase risk of and protect against the development of AMD and its subtypes. Differential expression of risk factors among populations suggests the need for a very personalized approach to the treatment of AMD and the development of genetic therapies. The SNP rs17563 plays a central role in many aspects of BMP4's association with AMD and may provide a novel therapeutic target for more than just AMD. The association of rs17563 with both subtypes of AMD and a host of other diseases makes it a promising target for a multidisease treatment regime that can be attuned to individuals based on their particular type of AMD, other AMD risk factors such as ROBO1 and RORA, and any other features of their disease profile. While BMP4 is by no means the decisive treatment target for AMD, this work showcases its potential in the realm of personalized medicine, especially in an ageing population.

OPLL

The research carried out here on the ossification of the posterior longitudinal ligament has challenged previous understandings of the prevalence and aspects of the disease in non-Asian populations. The results presented here provide a significant contribution to the research of this disease and have the potential to influence future inquiries into OPLL. The ability to study the disease in a large, skeletal collection has

allowed for new understanding that this disease is common in all populations and has a wide range of characteristics. Assumptions of low prevalence in Whites and Blacks, along with the distribution of ossification types and location were challenged while the notion of a male prevalence was confirmed in all groups. More research by both anthropologists and medical professionals can increase the understanding of OPLL in all populations, and collaboration between the two groups can define when OPLL becomes symptomatic and thus at what degree of ossification an informative prevalence study can be conducted. There is clearly more to this disease than has ever been considered before in the clinical literature, and understanding that about half of the population presents with some degree of ossification can help clinicians understand the true potential for OPLL-induced neurological complications as the population ages.

Forensic Identification Through Disease Association

Regardless of the eventual success or failure of the OPLL-BMP4-AMD identification method, the concept of trying to associate skeletal and other medical diseases for forensic identification through the utilization of disease-specific databases should be explored further. Medical patient databases are a valuable and underutilized resource in forensic identification, and anthropologists should strive to find ways to use them in identification, when appropriate. Collaboration between genetic and forensic researchers should be the ultimate goal. DNA and genetic research is the current gold standard for medico-legal cases and anthropologists should be embracing what genetics can add to identification instead of fearing that DNA will make anthropology obsolete.

The research presented here would only work with the combined support and knowledge of both anthropologists and medical professionals.

In order to find the genetic connections between diseases that can foster the use of these databases, it is important that while creating biological profiles, anthropologists note features that may not be considered individuating or traditionally helpful in identification, and research any connections between these features and diseases of current medical research interest. It is also important to consult the literature outside one's primary field, as it is surprising how little communication exists across disciplines. OPLL is a bone disease that has been of medical and clinical interest for over 40 years, with entire committees dedicated to clarifying every aspect of the disease. Yet, until this research it had never been looked at in a skeletal population and never considered, or in some cases even noticed, by anthropologists.

Due to the constraints of the hypothesis, the composition and size of the study cohorts, the repeatability of the analyses, the differential genetic results between AMD subtypes, and the prevalence of OPLL, the identification method proposed for this research could not be validated. Taking into account the issues that arose during analysis and the positive results of the research, there is promise that future work along with an alteration of the hypothesis could offer a manifestation of this project capable of being implemented as a recognized forensic identification technique.

Final Thoughts

The creation of any method, whether in anthropology, genetics, or forensics is not an easy task. Trying to develop an identification method drawing from all these

disciplines is even more complicated. At the commencement of this research, the goal was only to verify the association of OPLL and AMD through the SNP rs17563 and to use that association as a way to utilize medical patient databases to compile a pool of potential matches for identification. As this research progressed, the various aspects, such as BMP4 and OPLL, took on lives of their own. The depth of research into this gene and disease, which was required for this project, revealed a number of unexpected results greatly influential beyond the identification method itself.

In fact, BMP4 may be a therapeutic target for AMD, and a gene almost as influential as the RORA-ROBO1-HTRA1 triumvirate in the risk of acquiring the disease. Furthermore, the results of this study would suggest that OPLL, which has been extensively studied for decades, is much more prevalent than ever expected, and present in all world populations. This project will become the impetus for many more avenues of research than could have been predicted when the original research was undertaken. The method may not be applicable in its current form, but the knowledge gained from the process, the issues identified and addressed, the exposure to research outside of osteology, and the opportunity for more scientific inquiry based on these results, makes this project a successful endeavor into the expansion of general knowledge and the advancement of analytical investigation.

APPENDIX A

COMPLETE BMP4 ANALYSES

Table 6: Complete BMP4 SNP Analysis on All AMD Subtypes

				Statistics for each study*				
Model	**Study**	**Subgroup**	**Comparison**	**Odds**	**Lower**	**Upper**	**Z value**	**p value**
N=436	Greeks	AllAMD	rs1880add	1.048	0.618	1.776	0.174	0.8617
N=418	NESC	AllAMD (CLR)	rs1880add	0.232	0.018	2.957	-1.125	0.2606
Fixed				**0.985**	**0.588**	**1.651**	**-0.058**	**0.9539**
	Greeks	AllAMD	rs1880dom	1.105	0.624	1.956	0.343	0.7319
	NESC	AllAMD (CLR)	rs1880dom	0.232	0.018	2.957	-1.125	0.2606
Fixed				**1.025**	**0.587**	**1.79**	**0.088**	**0.93**
	Greeks	AllAMD	rs1880rec	0.473	0.042	5.342	-0.605	0.545
Fixed				**0.473**	**0.042**	**5.342**	**-0.605**	**0.545**
	Greeks	AllAMD	rs1884add	0.941	0.71	1.247	-0.423	0.6721
	NESC	AllAMD (CLR)	rs1884add	0.686	0.355	1.326	-1.12	0.2625
Fixed				**0.896**	**0.692**	**1.161**	**-0.829**	**0.4069**
	Greeks	AllAMD	rs1884dom	1.147	0.775	1.698	0.685	0.4931
	NESC	AllAMD (CLR)	rs1884dom	0.293	0.089	0.965	-2.018	0.0436
Fixed				**1.004**	**0.692**	**1.457**	**0.021**	**0.9836**
	Greeks	AllAMD	rs1884rec	0.591	0.334	1.045	-1.807	0.0707
	NESC	AllAMD (CLR)	rs1884rec	1.164	0.397	3.411	0.277	0.7819
Fixed				**0.686**	**0.414**	**1.135**	**-1.467**	**0.1424**
	Greeks	AllAMD	rs7563add	1.063	0.802	1.408	0.426	0.6705
N=1333	Koreans	AllAMD	rs7563add	1.098	0.909	1.326	0.971	0.3317
N=119	GSSibs	AllAMD (CLR)	rs7563add	0.644	0.192	2.161	-0.713	0.4761
	NESC	AllAMD (CLR)	rs7563add	1.704	1.037	2.801	2.103	0.0355
Fixed				**1.123**	**0.968**	**1.302**	**1.528**	**0.1266**
	Greeks	AllAMD	rs7563dom	1.209	0.761	1.921	0.803	0.4217
	Koreans	AllAMD	rs7563dom	1.159	0.725	1.853	0.616	0.5379
	GSSibs	AllAMD (CLR)	rs7563dom	0.752	0.154	3.667	-0.353	0.7244
	NESC	AllAMD (CLR)	rs7563dom	1.436	0.713	2.892	1.013	0.3109
Fixed				**1.206**	**0.9**	**1.617**	**1.252**	**0.2105**
	Greeks	AllAMD	rs7563rec	0.978	0.626	1.527	-0.098	0.9221
	Koreans	AllAMD	rs7563rec	1.113	0.88	1.408	0.892	0.3726
	GSSibs	AllAMD (CLR)	rs7563rec	0.438	0.05	3.82	-0.747	0.455
	NESC	AllAMD (CLR)	rs7563rec	2.762	1.207	6.319	2.406	0.0161
Fixed				**1.135**	**0.928**	**1.387**	**1.232**	**0.2178**
	Greeks	AllAMD	rs8159add	1.009	0.758	1.344	0.061	0.9512
	GSSibs	AllAMD (CLR)	rs8159add	0.432	0.113	1.647	-1.229	0.219
	NESC	AllAMD (CLR)	rs8159add	1.629	0.991	2.678	1.924	0.0543
Fixed				**1.101**	**0.863**	**1.406**	**0.773**	**0.4395**

* Results of meta-analysis under three different genetic models (additive, dominant, and recessive) for all AMD subtypes, after adjustment for age and sex. Significance in pink.

Table 6: Cont.

				Statistics for each study*				
Model	**Study**	**Subgroup**	**Comparison**	**Odds**	**Lower**	**Upper**	**Z value**	**p value**
	Greeks	AllAMD	rs8159dom	1.052	0.693	1.598	0.238	0.8121
	GSSibs	AllAMD (CLR)	rs8159dom	0.684	0.139	3.367	-0.467	0.6405
	NESC	AllAMD (CLR)	rs8159dom	1.621	0.808	3.251	1.36	0.1737
Fixed				**1.149**	**0.81**	**1.63**	**0.78**	**0.4355**
	Greeks	AllAMD	rs8159rec	0.952	0.569	1.593	-0.187	0.8514
	GSSibs	AllAMD (CLR)	rs8159rec	0.084	0.003	2.236	-1.479	0.1391
	NESC	AllAMD (CLR)	rs8159rec	2.25	0.925	5.472	1.788	0.0737
Fixed				**1.126**	**0.724**	**1.751**	**0.528**	**0.5972**
	Greeks	AllAMD	rs8820add	0.975	0.747	1.272	-0.186	0.8521
	Koreans	AllAMD	rs8820add	1.025	0.872	1.204	0.3	0.7642
	GSSibs	AllAMD (CLR)	rs8820add	0.604	0.185	1.97	-0.836	0.4032
	NESC	AllAMD (CLR)	rs8820add	0.733	0.464	1.157	-1.334	0.1821
Fixed				**0.979**	**0.858**	**1.116**	**-0.324**	**0.7456**
	Greeks	AllAMD	rs8820dom	1.104	0.708	1.721	0.437	0.6624
	Koreans	AllAMD	rs8820dom	1.148	0.89	1.481	1.062	0.288
	GSSibs	AllAMD (CLR)	rs8820dom	0.176	0.022	1.409	-1.637	0.1017
	NESC	AllAMD (CLR)	rs8820dom	0.65	0.312	1.353	-1.152	0.2495
Fixed				**1.065**	**0.863**	**1.315**	**0.589**	**0.556**
	Greeks	AllAMD	rs8820rec	0.852	0.553	1.313	-0.726	0.4678
	Koreans	AllAMD	rs8820rec	0.911	0.689	1.205	-0.653	0.514
	GSSibs	AllAMD (CLR)	rs8820rec	2.329	0.275	19.741	0.775	0.4382
	NESC	AllAMD (CLR)	rs8820rec	0.715	0.357	1.431	-0.948	0.3433
Fixed				**0.882**	**0.707**	**1.101**	**-1.11**	**0.267**
	Greeks	AllAMD	rs1880add	1.048	0.618	1.776	0.174	0.8617
	NESC	AllAMD (GEE)	rs1880add	1.241	0.484	3.184	0.449	0.6537
Fixed				**1.091**	**0.689**	**1.729**	**0.371**	**0.7105**
	Greeks	AllAMD	rs1880dom	1.105	0.624	1.956	0.343	0.7319
	NESC	AllAMD (GEE)	rs1880dom	1.184	0.432	3.244	0.329	0.7422
Fixed				**1.124**	**0.684**	**1.847**	**0.46**	**0.6454**
	Greeks	AllAMD	rs1880rec	0.473	0.042	5.342	-0.605	0.545
Fixed				**0.473**	**0.042**	**5.342**	**-0.605**	**0.545**
	Greeks	AllAMD	rs1884add	0.941	0.71	1.247	-0.423	0.6721
	NESC	AllAMD (GEE)	rs1884add	1.019	0.756	1.375	0.126	0.8994
Fixed				**0.977**	**0.796**	**1.199**	**-0.221**	**0.8247**

* Results of meta-analysis under three different genetic models (additive, dominant, and recessive) for all AMD subtypes, after adjustment for age and sex. Significance in pink.

Table 6: Cont.

				Statistics for each study*				
Model	**Study**	**Subgroup**	**Comparison**	**Odds**	**Lower**	**Upper**	**Z value**	**p value**
	Greeks	AllAMD	rs1884dom	1.147	0.775	1.698	0.685	0.4931
	NESC	AllAMD (GEE)	rs1884dom	1.013	0.662	1.55	0.061	0.9515
Fixed				**1.083**	**0.812**	**1.445**	**0.545**	**0.5857**
	Greeks	AllAMD	rs1884rec	0.591	0.334	1.045	-1.807	0.0707
	NESC	AllAMD (GEE)	rs1884rec	1.053	0.591	1.877	0.176	0.8606
Fixed				**0.786**	**0.524**	**1.179**	**-1.163**	**0.2447**
	Greeks	AllAMD	rs7563add	1.063	0.802	1.408	0.426	0.6705
	Koreans	AllAMD	rs7563add	1.098	0.909	1.326	0.971	0.3317
	GSSibs	AllAMD (GEE)	rs7563add	0.896	0.502	1.597	-0.373	0.7093
	NESC	AllAMD (GEE)	rs7563add	1.166	0.927	1.468	1.312	0.1894
Fixed				**1.1**	**0.97**	**1.248**	**1.481**	**0.1386**
	Greeks	AllAMD	rs7563dom	1.209	0.761	1.921	0.803	0.4217
	Koreans	AllAMD	rs7563dom	1.159	0.725	1.853	0.616	0.5379
	GSSibs	AllAMD (GEE)	rs7563dom	0.785	0.356	1.731	-0.599	0.549
	NESC	AllAMD (GEE)	rs7563dom	1.385	0.932	2.059	1.611	0.1071
Fixed				**1.208**	**0.949**	**1.538**	**1.534**	**0.125**
	Greeks	AllAMD	rs7563rec	0.978	0.626	1.527	-0.098	0.9221
	Koreans	AllAMD	rs7563rec	1.113	0.88	1.408	0.892	0.3726
	GSSibs	AllAMD (GEE)	rs7563rec	1.111	0.287	4.298	0.153	0.8784
	NESC	AllAMD (GEE)	rs7563rec	1.09	0.771	1.54	0.488	0.6257
Fixed				**1.085**	**0.909**	**1.294**	**0.9**	**0.3679**
	Greeks	AllAMD	rs8159add	1.009	0.758	1.344	0.061	0.9512
	GSSibs	AllAMD (GEE)	rs8159add	0.996	0.532	1.866	-0.011	0.991
	NESC	AllAMD (GEE)	rs8159add	1.175	0.92	1.502	1.291	0.1968
Fixed				**1.093**	**0.914**	**1.307**	**0.975**	**0.3295**
	Greeks	AllAMD	rs8159dom	1.052	0.693	1.598	0.238	0.8121
	GSSibs	AllAMD (GEE)	rs8159dom	0.963	0.432	2.151	-0.091	0.9277
	NESC	AllAMD (GEE)	rs8159dom	1.369	0.929	2.017	1.586	0.1127
Fixed				**1.181**	**0.904**	**1.544**	**1.219**	**0.223**

* Results of meta-analysis under three different genetic models (additive, dominant, and recessive) for all AMD subtypes, after adjustment for age and sex. Significance in pink.

Table 6: Cont.

				Statistics for each study*				
Model	**Study**	**Subgroup**	**Comparison**	**Odds**	**Lower**	**Upper**	**Z value**	**p value**
	Greeks	AllAMD	rs8159rec	0.952	0.569	1.593	-0.187	0.8514
	GSSibs	AllAMD (GEE)	rs8159rec	1.069	0.26	4.4	0.093	0.9259
	NESC	AllAMD (GEE)	rs8159rec	1.088	0.748	1.584	0.442	0.6582
Fixed				**1.04**	**0.773**	**1.399**	**0.261**	**0.794**
	Greeks	AllAMD	rs8820add	0.975	0.747	1.272	-0.186	0.8521
	Koreans	AllAMD	rs8820add	1.025	0.872	1.204	0.3	0.7642
	GSSibs	AllAMD (GEE)	rs8820add	0.654	0.347	1.232	-1.315	0.1886
	NESC	AllAMD (GEE)	rs8820add	0.962	0.768	1.206	-0.333	0.7392
Fixed				**0.984**	**0.876**	**1.104**	**-0.277**	**0.7819**
	Greeks	AllAMD	rs8820dom	1.104	0.708	1.721	0.437	0.6624
	Koreans	AllAMD	rs8820dom	1.148	0.89	1.481	1.062	0.288
	GSSibs	AllAMD (GEE)	rs8820dom	0.335	0.118	0.947	-2.063	0.0391
	NESC	AllAMD (GEE)	rs8820dom	0.929	0.643	1.341	-0.394	0.6936
Fixed				**1.038**	**0.862**	**1.25**	**0.391**	**0.6958**
	Greeks	AllAMD	rs8820rec	0.852	0.553	1.313	-0.726	0.4678
	Koreans	AllAMD	rs8820rec	0.911	0.689	1.205	-0.653	0.514
	GSSibs	AllAMD (GEE)	rs8820rec	0.95	0.34	2.652	-0.098	0.9218
	NESC	AllAMD (GEE)	rs8820rec	0.973	0.657	1.442	-0.136	0.8921
Fixed				**0.915**	**0.751**	**1.115**	**-0.881**	**0.3783**

* Results of meta-analysis under three different genetic models (additive, dominant, and recessive) for all AMD subtypes, after adjustment for age and sex. Significance in pink.

Table 7: Complete BMP4 SNP Analysis on Neovascular AMD

				Statistics for each study				
Model	**Study**	**Subgroup**	**Comparison**	**Odds**	**Lower**	**Upper**	**Z value**	**p value**
N=436	Greeks	NeoNorm	rs1880add	0.844	0.45	1.583	-0.528	0.5973
N=418	NESC	NeoNorm (CLR)	rs1880add	0.222	0.017	2.904	-1.147	0.2512
Fixed				**0.783**	**0.425**	**1.442**	**-0.786**	**0.4319**
	Greeks	NeoNorm	rs1880dom	0.828	0.413	1.661	-0.531	0.5952
	NESC	NeoNorm (CLR)	rs1880dom	0.222	0.017	2.904	-1.147	0.2512
Fixed				**0.757**	**0.386**	**1.482**	**-0.813**	**0.4163**
	Greeks	NeoNorm	rs1880rec	0.788	0.068	9.102	-0.191	0.8486
Fixed				**0.788**	**0.068**	**9.102**	**-0.191**	**0.8486**
	Greeks	NeoNorm	rs1884add	0.859	0.623	1.184	-0.928	0.3535
	NESC	NeoNorm (CLR)	rs1884add	0.634	0.307	1.309	-1.232	0.218
Fixed				**0.817**	**0.609**	**1.096**	**-1.347**	**0.1779**
	Greeks	NeoNorm	rs1884dom	1.05	0.672	1.64	0.214	0.8303
	NESC	NeoNorm (CLR)	rs1884dom	0.195	0.046	0.822	-2.227	0.0259
Fixed				**0.906**	**0.592**	**1.387**	**-0.455**	**0.6491**
	Greeks	NeoNorm	rs1884rec	0.465	0.23	0.94	-2.132	0.033
	NESC	NeoNorm (CLR)	rs1884rec	1.199	0.379	3.794	0.309	0.7575
Fixed				**0.602**	**0.33**	**1.097**	**-1.658**	**0.0973**
	Greeks	NeoNorm	rs7563add	1.167	0.848	1.607	0.947	0.3436
N=1333	Koreans	NeoNorm	rs7563add	1.005	0.807	1.252	0.045	0.9645
N=119	GSSibs	NeoNorm (CLR)	rs7563add	0.644	0.192	2.161	-0.713	0.4761
	NESC	NeoNorm (CLR)	rs7563add	1.447	0.828	2.53	1.296	0.1949
Fixed				**1.075**	**0.907**	**1.275**	**0.835**	**0.4038**
	Greeks	NeoNorm	rs7563dom	1.291	0.751	2.219	0.925	0.3552
	Koreans	NeoNorm	rs7563dom	1.112	0.645	1.917	0.382	0.7024
	GSSibs	NeoNorm (CLR)	rs7563dom	0.752	0.154	3.667	-0.353	0.7244
	NESC	NeoNorm (CLR)	rs7563dom	1.488	0.658	3.365	0.955	0.3398
Fixed				**1.218**	**0.867**	**1.71**	**1.139**	**0.2547**
	Greeks	NeoNorm	rs7563rec	1.172	0.712	1.93	0.624	0.5329
	Koreans	NeoNorm	rs7563rec	0.98	0.744	1.291	-0.144	0.8857
	GSSibs	NeoNorm (CLR)	rs7563rec	0.438	0.05	3.82	-0.747	0.455
	NESC	NeoNorm (CLR)	rs7563rec	1.786	0.677	4.709	1.172	0.241
Fixed				**1.045**	**0.828**	**1.319**	**0.371**	**0.7109**

* Results of meta-analysis under three different genetic models (additive, dominant, and recessive) for all AMD subtypes, after adjustment for age and sex. Significance in pink.

Table 7: Cont.

				Statistics for each study				
Model	**Study**	**Subgroup**	**Comparison**	**Odds**	**Lower**	**Upper**	**Z value**	**p value**
	Greeks	NeoNorm	rs8159add	1.171	0.848	1.618	0.958	0.3382
	GSSibs	NeoNorm (CLR)	rs8159add	0.432	0.113	1.647	-1.229	0.219
	NESC	NeoNorm (CLR)	rs8159add	1.523	0.871	2.663	1.476	0.1401
Fixed				**1.196**	**0.91**	**1.573**	**1.283**	**0.1994**
	Greeks	NeoNorm	rs8159dom	1.219	0.748	1.987	0.795	0.4269
	GSSibs	NeoNorm (CLR)	rs8159dom	0.684	0.139	3.367	-0.467	0.6405
	NESC	NeoNorm (CLR)	rs8159dom	1.843	0.819	4.148	1.477	0.1397
Fixed				**1.302**	**0.868**	**1.951**	**1.277**	**0.2017**
	Greeks	NeoNorm	rs8159rec	1.241	0.708	2.175	0.754	0.4506
	GSSibs	NeoNorm (CLR)	rs8159rec	0.084	0.003	2.236	-1.479	0.1391
	NESC	NeoNorm (CLR)	rs8159rec	1.553	0.575	4.197	0.868	0.3855
Fixed				**1.234**	**0.761**	**2.001**	**0.854**	**0.3932**
	Greeks	NeoNorm	rs8820add	0.903	0.667	1.223	-0.659	0.51
	Koreans	NeoNorm	rs8820add	0.922	0.761	1.117	-0.831	0.4057
	GSSibs	NeoNorm (CLR)	rs8820add	0.604	0.185	1.97	-0.836	0.4032
	NESC	NeoNorm (CLR)	rs8820add	0.784	0.464	1.325	-0.908	0.3636
Fixed				**0.898**	**0.77**	**1.047**	**-1.373**	**0.1697**
	Greeks	NeoNorm	rs8820dom	1.012	0.612	1.673	0.046	0.9629
	Koreans	NeoNorm	rs8820dom	1.14	0.845	1.537	0.859	0.3906
	GSSibs	NeoNorm (CLR)	rs8820dom	0.176	0.022	1.409	-1.637	0.1017
	NESC	NeoNorm (CLR)	rs8820dom	0.643	0.265	1.561	-0.976	0.329
Fixed				**1.034**	**0.809**	**1.321**	**0.264**	**0.7921**
	Greeks	NeoNorm	rs8820rec	0.748	0.45	1.243	-1.121	0.2623
	Koreans	NeoNorm	rs8820rec	0.645	0.452	0.92	-2.419	0.0156
	GSSibs	NeoNorm (CLR)	rs8820rec	2.329	0.275	19.74	0.775	0.4382
	NESC	NeoNorm (CLR)	rs8820rec	0.824	0.378	1.795	-0.487	0.6261
Fixed				**0.707**	**0.54**	**0.927**	**-2.51**	**0.0121**
	Greeks	NeoNorm	rs1880add	0.844	0.45	1.583	-0.528	0.5973
	NESC	NeoNorm(GEE)	rs1880add	1.33	0.476	3.718	0.544	0.5866
Fixed				**0.955**	**0.559**	**1.634**	**-0.167**	**0.8676**

* Results of meta-analysis under three different genetic models (additive, dominant, and recessive) for all AMD subtypes, after adjustment for age and sex. Significance in pink.

Table 7: Cont.

				Statistics for each study				
Model	**Study**	**Subgroup**	**Comparison**	**Odds**	**Lower**	**Upper**	**Z value**	**p value**
	Greeks	NeoNorm	rs1880dom	0.828	0.413	1.661	-0.531	0.5952
	NESC	NeoNorm (GEE)	rs1880dom	1.33	0.476	3.718	0.544	0.5866
Fixed				**0.961**	**0.54**	**1.71**	**-0.135**	**0.8925**
	Greeks	NeoNorm	rs1880rec	0.788	0.068	9.102	-0.191	0.8486
Fixed				**0.788**	**0.068**	**9.102**	**-0.191**	**0.8486**
	Greeks	NeoNorm	rs1884add	0.859	0.623	1.184	-0.928	0.3535
	NESC	NeoNorm (GEE)	rs1884add	1.042	0.781	1.392	0.282	0.7779
Fixed				**0.956**	**0.771**	**1.185**	**-0.411**	**0.6809**
	Greeks	NeoNorm	rs1884dom	1.05	0.672	1.64	0.214	0.8303
	NESC	NeoNorm (GEE)	rs1884dom	0.995	0.665	1.487	-0.026	0.9794
Fixed				**1.019**	**0.756**	**1.374**	**0.124**	**0.9011**
	Greeks	NeoNorm	rs1884rec	0.465	0.23	0.94	-2.132	0.033
	NESC	NeoNorm (GEE)	rs1884rec	1.195	0.667	2.14	0.599	0.5493
Fixed				**0.814**	**0.52**	**1.275**	**-0.898**	**0.3691**
	Greeks	NeoNorm	rs7563add	1.167	0.848	1.607	0.947	0.3436
	Koreans	NeoNorm	rs7563add	1.005	0.807	1.252	0.045	0.9645
	GSSibs	NeoNorm (GEE)	rs7563add	0.896	0.502	1.597	-0.373	0.7093
	NESC	NeoNorm (GEE)	rs7563add	2.114	1.702	2.627	6.762	0~
Fixed				**1.369**	**1.196**	**1.567**	**4.552**	**0~**
	Greeks	NeoNorm	rs7563dom	1.291	0.751	2.219	0.925	0.3552
	Koreans	NeoNorm	rs7563dom	1.112	0.645	1.917	0.382	0.7024
	GSSibs	NeoNorm (GEE)	rs7563dom	0.785	0.356	1.731	-0.599	0.549
	NESC	NeoNorm (GEE)	rs7563dom	1.392	0.921	2.103	1.571	0.1162
Fixed				**1.216**	**0.933**	**1.584**	**1.446**	**0.1482**
	Greeks	NeoNorm	rs7563rec	1.172	0.712	1.93	0.624	0.5329
	Koreans	NeoNorm	rs7563rec	0.98	0.744	1.291	-0.144	0.8857
	GSSibs	NeoNorm (GEE)	rs7563rec	1.111	0.287	4.298	0.153	0.8784
	NESC	NeoNorm (GEE)	rs7563rec	3.261	2.265	4.695	6.357	0~
Fixed				**1.447**	**1.186**	**1.765**	**3.638**	**0.0003**

* Results of meta-analysis under three different genetic models (additive, dominant, and recessive) for all AMD subtypes, after adjustment for age and sex. Significance in pink. ~P-value is not actually significant; it is an error of convergence in SAS.

Table 7: Cont.

				Statistics for each study				
Model	**Study**	**Subgroup**	**Comparison**	**Odds**	**Lower**	**Upper**	**Z value**	**p value**
	Greeks	NeoNorm	rs8159add	1.171	0.848	1.618	0.958	0.3382
	GSSibs	NeoNorm (GEE)	rs8159add	0.996	0.532	1.866	-0.011	0.991
	NESC	NeoNorm (GEE)	rs8159add	2.488	2.144	2.886	12.036	0
Fixed				**2.107**	**1.847**	**2.404**	**11.081**	**0**
	Greeks	NeoNorm	rs8159dom	1.219	0.748	1.987	0.795	0.4269
	GSSibs	NeoNorm (GEE)	rs8159dom	0.963	0.432	2.151	-0.091	0.9277
	NESC	NeoNorm (GEE)	rs8159dom	1.686	1.036	2.744	2.102	0.0355
Fixed				**1.348**	**0.982**	**1.851**	**1.848**	**0.0646**
	Greeks	NeoNorm	rs8159rec	1.241	0.708	2.175	0.754	0.4506
	GSSibs	NeoNorm (GEE)	rs8159rec	1.069	0.26	4.4	0.093	0.9259
	NESC	NeoNorm (GEE)	rs8159rec	2.053	1.035	4.071	2.059	0.0395
Fixed				**1.474**	**0.973**	**2.232**	**1.833**	**0.0669**
	Greeks	NeoNorm	rs8820add	0.903	0.667	1.223	-0.659	0.51
	Koreans	NeoNorm	rs8820add	0.922	0.761	1.117	-0.831	0.4057
	GSSibs	NeoNorm (GEE)	rs8820add	0.654	0.347	1.232	-1.315	0.1886
	NESC	NeoNorm (GEE)	rs8820add	1.623	1.127	2.337	2.605	0.0092
Fixed				**0.985**	**0.853**	**1.137**	**-0.208**	**0.8351**
	Greeks	NeoNorm	rs8820dom	1.012	0.612	1.673	0.046	0.9629
	Koreans	NeoNorm	rs8820dom	1.14	0.845	1.537	0.859	0.3906
	GSSibs	NeoNorm (GEE)	rs8820dom	0.335	0.118	0.947	-2.063	0.0391
	NESC	NeoNorm (GEE)	rs8820dom	1.944	1.042	3.625	2.091	0.0366
Fixed				**1.126**	**0.893**	**1.42**	**1.004**	**0.3153**
	Greeks	NeoNorm	rs8820rec	0.748	0.45	1.243	-1.121	0.2623
	Koreans	NeoNorm	rs8820rec	0.645	0.452	0.92	-2.419	0.0156
	GSSibs	NeoNorm (GEE)	rs8820rec	0.95	0.34	2.652	-0.098	0.9218
	NESC	NeoNorm (GEE)	rs8820rec	2.308	1.16	4.592	2.383	0.0172
Fixed				**0.824**	**0.636**	**1.068**	**-1.465**	**0.143**

* Results of meta-analysis under three different genetic models (additive, dominant, and recessive) for all AMD subtypes, after adjustment for age and sex. Significance in pink.

Table 8: Complete BMP4 SNP Analysis on Dry AMD

				Statistics for each study				
Model	**Study**	**Subgroup**	**Comparison**	**Odds**	**Lower**	**Upper**	**Z-Value**	**p-Value**
N=436	Greeks	DryNorm	rs1880add	1.594	0.815	3.118	1.362	0.1732
Fixed				**1.594**	**0.815**	**3.118**	**1.362**	**0.1732**
	Greeks	DryNorm	rs1880dom	1.845	0.898	3.791	1.667	0.0955
Fixed				**1.845**	**0.898**	**3.791**	**1.667**	**0.0955**
	Greeks	DryNorm	rs1884add	1.024	0.689	1.521	0.117	0.9065
Fixed				**1.024**	**0.689**	**1.521**	**0.117**	**0.9065**
	Greeks	DryNorm	rs1884dom	1.254	0.701	2.243	0.763	0.4454
Fixed				**1.254**	**0.701**	**2.243**	**0.763**	**0.4454**
	Greeks	DryNorm	rs1884rec	0.712	0.311	1.63	-0.804	0.4213
Fixed				**0.712**	**0.311**	**1.63**	**-0.804**	**0.4213**
	Greeks	DryNorm	rs7563add	0.959	0.637	1.445	-0.2	0.8413
N=1333	Koreans	DryNorm	rs7563add	1.46	1.073	1.987	2.408	0.0161
N=418	NESC	DryNorm (CLR)	rs7563add	4.204	0.668	26.45	1.53	0.126
Fixed				**1.281**	**1.004**	**1.636**	**1.991**	**0.0464**
	Greeks	DryNorm	rs7563dom	1.286	0.648	2.553	0.719	0.4721
	Koreans	DryNorm	rs7563dom	1.394	0.64	3.037	0.836	0.403
	NESC	DryNorm (CLR)	rs7563dom	0.956	0.134	6.827	-0.045	0.9642
Fixed				**1.304**	**0.793**	**2.145**	**1.045**	**0.2959**
	Greeks	DryNorm	rs7563rec	0.688	0.34	1.392	-1.04	0.2983
	Koreans	DryNorm	rs7563rec	1.631	1.126	2.363	2.585	0.0097
Fixed				**1.353**	**0.974**	**1.878**	**1.804**	**0.0713**
	Greeks	DryNorm	rs8159add	0.773	0.505	1.184	-1.184	0.2362
	NESC	DryNorm (CLR)	rs8159add	2	0.341	11.73	0.768	0.4426
Fixed				**0.814**	**0.538**	**1.232**	**-0.972**	**0.3311**
	Greeks	DryNorm	rs8159dom	0.846	0.471	1.521	-0.559	0.5762
	NESC	DryNorm (CLR)	rs8159dom	0.66	0.084	5.194	-0.395	0.693
Fixed				**0.83**	**0.472**	**1.46**	**-0.646**	**0.5186**
	Greeks	DryNorm	rs8159rec	0.506	0.202	1.266	-1.456	0.1455
Fixed				**0.506**	**0.202**	**1.266**	**-1.456**	**0.1455**
	Greeks	DryNorm	rs8820add	1.082	0.739	1.584	0.405	0.6853
	Koreans	DryNorm	rs8820add	1.184	0.928	1.511	1.358	0.1744
	NESC	DryNorm (CLR)	rs8820add	0.632	0.15	2.665	-0.625	0.532
Fixed				**1.14**	**0.93**	**1.397**	**1.261**	**0.2075**

* Results of meta-analysis under three different genetic models (additive, dominant, and recessive) for all AMD subtypes, after adjustment for age and sex. Significance in pink.

Table 8: Cont.

				Statistics for each study				
Model	**Study**	**Subgroup**	**Comparison**	**Odds**	**Lower**	**Upper**	**Z-Value**	**p-Value**
	Greeks	DryNorm	rs8820dom	1.282	0.654	2.514	0.723	0.4696
	Koreans	DryNorm	rs8820dom	1.171	0.788	1.741	0.78	0.4354
	NESC	DryNorm (CLR)	rs8820dom	0.537	0.075	3.853	-0.618	0.5363
Fixed				**1.171**	**0.836**	**1.64**	**0.918**	**0.3585**
	Greeks	DryNorm	rs8820rec	0.989	0.536	1.825	-0.035	0.9718
	Koreans	DryNorm	rs8820rec	1.363	0.911	2.04	1.505	0.1323
	NESC	DryNorm (CLR)	rs8820rec	0.774	0.09	6.646	-0.234	0.8154
Fixed				**1.223**	**0.877**	**1.706**	**1.187**	**0.2354**
	Greeks	DryNorm	rs1880add	1.594	0.815	3.118	1.362	0.1732
	NESC	DryNorm (GEE)	rs1880add	0.945	0.244	3.654	-0.082	0.9349
Fixed				**1.438**	**0.788**	**2.622**	**1.184**	**0.2364**
	Greeks	DryNorm	rs1880dom	1.845	0.898	3.791	1.667	0.0955
	NESC	DryNorm (GEE)	rs1880dom	0.615	0.13	2.902	-0.614	0.5394
Fixed				**1.519**	**0.79**	**2.918**	**1.254**	**0.21**
	Greeks	DryNorm	rs1884add	1.024	0.689	1.521	0.117	0.9065
	NESC	DryNorm (GEE)	rs1884add	1.14	0.662	1.962	0.473	0.6362
Fixed				**1.063**	**0.772**	**1.464**	**0.374**	**0.7087**
	Greeks	DryNorm	rs1884dom	1.254	0.701	2.243	0.763	0.4454
	NESC	DryNorm (GEE)	rs1884dom	1.326	0.601	2.925	0.698	0.4853
Fixed				**1.279**	**0.8**	**2.043**	**1.028**	**0.3039**
	Greeks	DryNorm	rs1884rec	0.712	0.311	1.63	-0.804	0.4213
	NESC	DryNorm (GEE)	rs1884rec	0.84	0.342	2.06	-0.381	0.7029
Fixed				**0.768**	**0.418**	**1.412**	**-0.85**	**0.3956**
	Greeks	DryNorm	rs7563add	0.959	0.637	1.445	-0.2	0.8413
	Koreans	DryNorm	rs7563add	1.46	1.073	1.987	2.408	0.0161
	NESC	DryNorm (GEE)	rs7563add	1.082	0.779	1.503	0.47	0.6385
Fixed				**1.189**	**0.977**	**1.449**	**1.726**	**0.0844**
	Greeks	DryNorm	rs7563dom	1.286	0.648	2.553	0.719	0.4721
	Koreans	DryNorm	rs7563dom	1.394	0.64	3.037	0.836	0.403
	NESC	DryNorm (GEE)	rs7563dom	1.225	0.715	2.098	0.738	0.4606
Fixed				**1.28**	**0.882**	**1.856**	**1.299**	**0.1938**

* Results of meta-analysis under three different genetic models (additive, dominant, and recessive) for all AMD subtypes, after adjustment for age and sex. Significance in pink.

Table 8: Cont.

				Statistics for each study				
Model	**Study**	**Subgroup**	**Comparison**	**Odds**	**Lower**	**Upper**	**Z-Value**	**p-Value**
	Greeks	DryNorm	rs7563rec	0.688	0.34	1.392	-1.04	0.2983
	Koreans	DryNorm	rs7563rec	1.631	1.126	2.363	2.585	0.0097
	NESC	DryNorm (GEE)	rs7563rec	0.989	0.549	1.781	-0.037	0.9705
Fixed				**1.256**	**0.943**	**1.672**	**1.557**	**0.1194**
	Greeks	DryNorm	rs8159add	0.773	0.505	1.184	-1.184	0.2362
	NESC	DryNorm (GEE)	rs8159add	0.961	0.69	1.338	-0.235	0.8141
Fixed				**0.885**	**0.682**	**1.15**	**-0.912**	**0.3616**
	Greeks	DryNorm	rs8159dom	0.846	0.471	1.521	-0.559	0.5762
	NESC	DryNorm (GEE)	rs8159dom	1.047	0.632	1.736	0.178	0.8587
Fixed				**0.956**	**0.652**	**1.402**	**-0.23**	**0.818**
	Greeks	DryNorm	rs8159rec	0.506	0.202	1.266	-1.456	0.1455
	NESC	DryNorm (GEE)	rs8159rec	0.808	0.425	1.537	-0.65	0.5156
Fixed				**0.692**	**0.409**	**1.172**	**-1.368**	**0.1713**
	Greeks	DryNorm	rs8820add	1.082	0.739	1.584	0.405	0.6853
	Koreans	DryNorm	rs8820add	1.184	0.928	1.511	1.358	0.1744
	NESC	DryNorm (GEE)	rs8820add	0.979	0.698	1.374	-0.121	0.9038
Fixed				**1.104**	**0.926**	**1.316**	**1.103**	**0.2702**
	Greeks	DryNorm	rs8820dom	1.282	0.654	2.514	0.723	0.4696
	Koreans	DryNorm	rs8820dom	1.171	0.788	1.741	0.78	0.4354
	NESC	DryNorm (GEE)	rs8820dom	1.003	0.58	1.734	0.011	0.9911
Fixed				**1.14**	**0.853**	**1.524**	**0.887**	**0.375**
	Greeks	DryNorm	rs8820rec	0.989	0.536	1.825	-0.035	0.9718
	Koreans	DryNorm	rs8820rec	1.363	0.911	2.04	1.505	0.1323
	NESC	DryNorm (GEE)	rs8820rec	0.943	0.527	1.685	-0.2	0.8416
Fixed				**1.155**	**0.863**	**1.546**	**0.97**	**0.3319**

* Results of meta-analysis under three different genetic models (additive, dominant, and recessive) for all AMD subtypes, after adjustment for age and sex. Significance in pink.

Table 9: Complete Cases-only Interaction Analysis

Cases Only - All Cohorts	All AMD				Neo AMD				Dry AMD			
Interaction*	Odds Ratio	95% Low	95% High	p value	Odds Ratio	95% Low	95% High	p value	Odds Ratio	95% Low	95% High	p value
ARMS2 rs10490924* BMP4 rs12898159	1.011	0.837	1.222	0.9065	1.035	0.828	1.292	0.7637	0.86	0.579	1.277	0.4544
HTRA1 rs11200638* BMP4 rs12898159	1.023	0.849	1.233	0.8124	1.074	0.864	1.336	0.5186	0.828	0.556	1.234	0.3542
HTRA1 rs1049331* BMP4 rs12898159	1.02	0.851	1.222	0.8296	1.053	0.85	1.306	0.6357	0.857	0.59	1.244	0.4155
RORA rs730754* BMP4 rs12898159	0.89	0.725	1.091	0.2617	0.84	0.656	1.077	0.1687	1.181	0.796	1.753	0.4089
RORA rs12900948* BMP4 rs12898159	0.911	0.743	1.117	0.3711	0.866	0.674	1.112	0.2585	1.111	0.751	1.645	0.5974
RORA rs8034864* BMP4 rs12898159	0.832	0.658	1.052	0.124	0.714	0.538	0.948	0.0197	1.43	0.892	2.292	0.1372
RORA rs4335725* BMP4 rs12898159	1.024	0.809	1.296	0.8452	1.084	0.822	1.428	0.5675	0.864	0.525	1.422	0.5657
ROBO1 rs1387665* BMP4 rs12898159	0.811	0.664	0.99	0.0395	0.741	0.585	0.939	0.0133	0.964	0.636	1.459	0.8614
ROBO1 rs4513416* BMP4 rs12898159	1.242	1.017	1.518	0.034	1.409	1.11	1.789	0.0048	1.01	0.665	1.533	0.9634
ROBO1 rs9309833* BMP4 rs12898159	0.872	0.677	1.125	0.2919	0.856	0.623	1.175	0.3355	0.819	0.512	1.311	0.4058
ARMS2 rs10490924* BMP4 rs17563	1.197	1.028	1.393	0.0206	1.18	0.978	1.424	0.0838	1.252	0.938	1.67	0.1268
HTRA1 rs11200638* BMP4 rs17563	1.275	1.097	1.483	0.0016	1.211	1.007	1.456	0.0422	1.417	1.06	1.894	0.0186
HTRA1 rs1049331* BMP4 rs17563	1.246	1.073	1.446	0.004	1.183	0.984	1.422	0.0734	1.37	1.028	1.824	0.0314

* Summary of significant cases-only interaction analysis between the BMP4 SNPs and SNPs from the RORA-ROBO1-HTRA1 triumvirate among all cases and wet (Neo) and dry cases specifically (significant interactions are in pink).

Table 9: Cont.

Cases Only - All Cohorts	All AMD				Neo AMD				Dry AMD			
Interaction*	Odds Ratio	95% Low	95% High	p value	Odds Ratio	95% Low	95% High	p value	Odds Ratio	95% Low	95% High	p value
RORA rs730754* BMP4 rs17563	1.32	1.136	1.533	0.0003	1.163	0.967	1.398	0.1086	1.928	1.432	2.596	1.51E-05
RORA rs12900948* BMP4 rs17563	1.156	0.995	1.344	0.0582	1.095	0.909	1.32	0.3389	1.343	1.004	1.797	0.0467
RORA rs8034864* BMP4 rs17563	1.474	1.27	1.711	3.47E-07	1.267	1.055	1.522	0.0114	2.438	1.798	3.307	9.78E-09
RORA rs4335725* BMP4 rs17563	0.982	0.775	1.244	0.8802	1.038	0.788	1.367	0.7928	0.884	0.532	1.468	0.6328
ROBO1 rs1387665* BMP4 rs17563	0.76	0.653	0.885	0.0004	0.71	0.591	0.853	0.0003	0.853	0.627	1.16	0.3098
ROBO1 rs4513416* BMP4 rs17563	1.268	1.089	1.477	0.0023	1.399	1.161	1.687	0.0004	1.096	0.813	1.478	0.5455
ROBO1 rs9309833* BMP4 rs17563	1.13	0.953	1.339	0.1595	1.121	0.902	1.394	0.3037	0.973	0.715	1.323	0.8609
ARMS2 rs10490924* BMP4 rs2761880	1.04	0.693	1.562	0.8497	1.229	0.733	2.063	0.434	0.943	0.454	1.96	0.8751
HTRA1 rs11200638* BMP4 rs2761880	1.043	0.7	1.555	0.8363	1.192	0.721	1.97	0.4928	0.959	0.458	2.007	0.9113
HTRA1 rs1049331* BMP4 rs2761880	0.979	0.667	1.436	0.9131	1.122	0.686	1.835	0.6466	0.83	0.414	1.662	0.5981
RORA rs730754* BMP4 rs2761880	1.146	0.72	1.825	0.5652	1.194	0.634	2.25	0.5823	0.933	0.455	1.914	0.8499
RORA rs12900948* BMP4 rs2761880	1.015	0.635	1.62	0.9515	1.033	0.549	1.945	0.9196	0.765	0.367	1.595	0.4755
RORA rs8034864* BMP4 rs2761880	1.099	0.671	1.801	0.7079	1.591	0.856	2.958	0.1422	0.434	0.136	1.391	0.1602

* Summary of significant cases-only interaction analysis between the BMP4 SNPs and SNPs from the RORA-ROBO1-HTRA1 triumvirate among all cases and wet (Neo) and dry cases specifically (significant interactions are in pink).

Table 9: Cont.

Cases Only - All Cohorts	All AMD				Neo AMD				Dry AMD			
Interaction*	**Odds ratio**	**95% Low**	**95% High**	**p value**	**Odds ratio**	**95% Low**	**95% High**	**p value**	**Odds ratio**	**95% Low**	**95% High**	**p value**
RORA rs4335725* BMP4 rs2761880	0.841	0.504	1.403	0.5073	0.722	0.367	1.423	0.3469	1.372	0.592	3.178	0.4611
ROBO1 rs1387665* BMP4 rs2761880	1.051	0.673	1.641	0.8282	1.014	0.578	1.781	0.9609	1.193	0.542	2.629	0.6607
ROBO1 rs4513416* BMP4 rs2761880	1.068	0.685	1.667	0.7714	0.977	0.549	1.736	0.9362	1.284	0.605	2.726	0.515
ROBO1 rs9309833* BMP4 rs2761880	1.128	0.647	1.968	0.6709	1.513	0.742	3.086	0.2545	0.821	0.313	2.156	0.6892
ARMS2 rs10490924* BMP4 rs2761884	1.068	0.858	1.329	0.5581	1.061	0.821	1.371	0.6509	1.148	0.712	1.85	0.5715
HTRA1 rs11200638* BMP4 rs2761884	1.022	0.824	1.268	0.8417	1.008	0.785	1.295	0.9477	1.148	0.708	1.862	0.576
HTRA1 rs1049331* BMP4 rs2761884	0.99	0.805	1.216	0.9211	0.996	0.78	1.272	0.9724	1.015	0.652	1.58	0.9477
RORA rs730754* BMP4 rs2761884	1.058	0.826	1.356	0.6548	1.102	0.811	1.498	0.5328	0.933	0.583	1.494	0.7735
RORA rs12900948* BMP4 rs2761884	1.032	0.806	1.322	0.8029	1.086	0.801	1.474	0.5953	0.994	0.619	1.597	0.9805
RORA rs8034864* BMP4 rs2761884	1.154	0.878	1.518	0.3052	1.182	0.844	1.656	0.3305	1.087	0.642	1.84	0.7556
RORA rs4335725* BMP4 rs2761884	1.207	0.927	1.573	0.1623	1.251	0.917	1.707	0.1575	1.051	0.596	1.851	0.8641
ROBO1 rs1387665* BMP4 rs2761884	1.424	1.115	1.817	0.0046	1.303	0.975	1.742	0.0735	1.685	1.018	2.79	0.0426
ROBO1 rs4513416* BMP4 rs2761884	0.753	0.592	0.959	0.0213	0.683	0.512	0.912	0.0098	0.976	0.601	1.586	0.9232

* Summary of significant cases-only interaction analysis between the BMP4 SNPs and SNPs from the RORA-ROBO1-HTRA1 triumvirate among all cases and wet (Neo) and dry cases specifically (significant interactions are in pink).

Table 9: Cont.

Cases Only - All Cohorts	All AMD				Neo AMD				Dry AMD			
Interaction*	**Odds ratio**	**95% Low**	**95% High**	**p value**	**Odds ratio**	**95% Low**	**95% High**	**p value**	**Odds ratio**	**95% Low**	**95% High**	**p value**
ROBO1 rs9309833* BMP4 rs2761884	0.91	0.667	1.241	0.5509	1.025	0.689	1.525	0.9035	0.905	0.516	1.588	0.7277
ARMS2 rs10490924* BMP4 rs4898820	0.94	0.806	1.096	0.4287	0.984	0.812	1.192	0.8669	0.855	0.641	1.141	0.2868
HTRA1 rs11200638* BMP4 rs4898820	0.933	0.801	1.087	0.3742	0.982	0.812	1.186	0.8471	0.805	0.603	1.075	0.1419
HTRA1 rs1049331* BMP4 rs4898820	0.942	0.81	1.095	0.4331	1.001	0.829	1.208	0.992	0.78	0.588	1.035	0.0847
RORA rs730754* BMP4 rs4898820	1.022	0.88	1.187	0.778	1.107	0.918	1.334	0.2868	0.731	0.549	0.973	0.0318
RORA rs12900948* BMP4 rs4898820	0.98	0.843	1.14	0.7968	1.075	0.89	1.298	0.4547	0.745	0.559	0.992	0.0436
RORA rs8034864* BMP4 rs4898820	1.025	0.884	1.187	0.7478	1.109	0.921	1.334	0.2739	0.789	0.598	1.041	0.0939
RORA rs4335725* BMP4 rs4898820	1.063	0.84	1.345	0.6107	1.117	0.846	1.475	0.4364	1.094	0.808	1.481	0.563
ROBO1 rs1387665* BMP4 rs4898820	1.131	0.972	1.316	0.1124	1.188	0.987	1.43	0.0679	0.996	0.613	1.619	0.9887
ROBO1 rs4513416* BMP4 rs4898820	0.932	0.8	1.086	0.3661	0.843	0.699	1.016	0.073	1.113	0.825	1.5	0.4832
ROBO1 rs9309833* BMP4 rs4898820	1.009	0.851	1.196	0.9203	0.952	0.763	1.187	0.6603	1.104	0.816	1.495	0.5208

* Summary of significant cases-only interaction analysis between the BMP4 SNPs and SNPs from the RORA-ROBO1-HTRA1 triumvirate among all cases and wet (Neo) and dry cases specifically (significant interactions are in pink).

APPENDIX B

OPLL RECORDING SHEET

Table 10: Example of OPLL Recording Sheet

Collection Prevalence

Number	From File Extent	Sex	Race	Age	OPLL?	Location	Type	At Max Length	Width	Depth
715		F	WH	78	Y	C3-7	S	13.15	3.7	1.37
1433	R	F	WH	78	Y	C2-6	S	13.03	5.08	1.59
1219		M	WH	79	Y	C3-5	S	12.92	4.2	1.12
1461		F	BL	80	Y	C3-6	S	11.45	4.71	1.09
1445		M	BL	80	Y	C2-7	M	16.95	5.24	1.9
1635		F	WH	81	Y	C2-7	S	11.1	3.91	2.05
1502	R	F	WH	82	Y	C3-4	S	15.7	3.43	1.55
1545		M	WH	82	Y	C3-4	S	14.7	3.51	1.39
76	R	F	WH	62	Y	C3-4	S	11.14	3.45	1
951	R	F	WH	62	Y	C3-4	C	13.25	4.48	2.35
726		M	BL	62	Y	C3-5	S	11.84	4.23	1.21
503		M	BL	76	Y	C3-6	S	14.53	6	1.96
278		M	BL	76	Y	C2-6	M	12.53	8.92	2.13
100	RR	F	BL	85	Y	C3-6	S	12.51	5.09	2
1117		M	BL	85	Y	C3-5	S	12.32	5.35	1.91
748		M	WH	85	Y	C3-5	S	14	5.22	1.13
979		M	WH	85	Y	C2-4	S	14.1	4.43	1.13

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